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Responses of *Phalaris canariensis* L. Exposed to Commercial Fuels during Growth

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Abstract The growth behavior of canary grass (*Phalaris canariensis L*) when cultivated in presence of farming fuels is reported in this work. *P. canariensis* L. is relevant in several countries. It is an emergent plant for phytoremediation and biofuel activities. The following variables: root length, stem length, total plant weight, green tissue weight (tiller, leaf), and total chlorophyll and chlorophyll a/b ratio, were monitored during the growth in presence of commercial fuels (premium grade, regular grade, diesel, and kerosene) at different concentrations. We applied a comprehensive statistical analysis to understand the results: Univariate analysis, factorial analysis of variance, and subsequent Tukey test were applied to the variables to assess the significance of the differences found. The normality of these variables was analyzed with the Shapiro Wilk test. All parameters were affected by all type and concentrations of fuels and its interaction. This is one of the first reported cases which describe the growth parameters responses from canary grass when cultivated in presence of an essentially constant concentration of farming fuels.

Keywords Fuels · Toxicity · Canary grass · *Phalaris canariensis*

Capsule *P. canariensis* L, an emergent plant for phytoremediation and biofuel activities, is affected by commercial fuels usually present in fuel spills at farms.

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1 Introduction

Farming activities usually release hydrocarbon fuel due to accidental spills. Sometimes, these contaminants do not result in hazardous waste formation but reduce the overall productivity of the farmed area. Based on agricultural needs, many remedial programs implemented by government agencies are over-designed, expensive, and have little or no clean-closure criteria.

Farming operations require use of gas and diesel-powered machines for tillage, planting application, harvesting, and transportation equipment. When groundwater is pumped from under the farmland, liquid-fuel internal combustion engines are frequently used to power the pump. These engines are refueled in the field from portable or moveable supply tanks. Invariably, liquid-fueling operations result in releases of liquid hydrocarbons (Deuel and Holliday 1997; Holliday and Deuel Jr 2006; Pons Jiménez 2010). Because of its short-chain hydrocarbons, diesel fuel acts as an herbicide, lowering the agronomic potential of the impacted area (Miller and Honarvar 1975; MacKinnon and Duncan 2013). It is responsible for hydrocarbon wastes produced in certain places and contaminates large parts of soil, groundwater, and surface and deep water (Kirchmann and Thorvaldsson 2000; De viana et al. 2003). In addition, if the soil has been continuously contaminated, the levels and variety of microorganisms in the soil will be diminished to the point in which the remaining bacterial populations will not facilitate contaminant degradation and/or enhance plant growth (Burd et al. 1998; Glick 1995; Balwin 1922; Huang et al. 2004; Siciliano and Germida 1997).

P. canariensis L. is the only member of its genus used as feed for caged birds. It was also used as sizing agent for some textiles, and the canary grass farinaceous food is blended with wheat flour for bread-making in some Southwestern European countries (Oram 2004). The area planted annually in Argentina is relatively important. Argentina is among the largest exporters (Yagüez 2002). The augmented use of biofuels and more particularly biodiesel in Europe has increased the interest in including *P. canariensis* L. as raw material.

P. canariensis is still cultivated to a limited extent as a food grain in several countries (Oram 2004). It is used as hypolipemiant (blood lipid reducer), and has demulcent properties (it has the effect of acting as a protective barrier on irritated or inflamed tissues). In the Canarias Islands, it is considered a great remedy for kidney and bladder diseases. It is indicated for prevention of hypercholesterolemia and atherosclerosis, and in the following situations that require increased diuresis: genitourinary disorders (cystitis), hyperazotemia, hyperuricemia, gout, hypertension, edema, and overweight accompanied by liquid retention (Concepción 1999).

P. canariensis has been considered for use in phytoremediation technologies (Schnoor 1997; Adam and Duncan 2002). Due to the high possibilities of finding soils with fuel contaminants caused by farming operations, its negative economic effects on plant yield and the lack of attention on the canary grass behavior under stress, more *P. canariensis* growth studies are necessary. Normally, fuels are held in the surface soil and within the rooting zone of most plant species (Adam and Duncan 2002). Polycyclic aromatic hydrocarbons (PAHs) can penetrate through the cell membranes, decrease water and nutrient utilization efficiency, and inhibit photosynthetic activity and electron transport (Nakata et al. 2011; Ma et al. 2010). Therefore, some physiological characteristics can be used as indicators for tolerance to contamination (Huang et al. 2004). These traits include the following (but are not limited to): germination; rapid growth; vigorous root systems; and the ability to maintain water content, chlorophyll levels, and the chlorophyll a/b ratio. Recently, MacKinnon and Duncan (2013) found that relatively low levels of diesel fuel contamination caused delayed shoot/root emergence and reduced germination in *P. canariensis*, but no information is given related to its growth under persistent contamination conditions.

In this study, *P. canariensis* L. seeds were grown in a wet support with an essentially constant concentration of commercial fuels (premium grade fuel, regular grade fuel, diesel, and kerosene). Several parameters of the plants were monitored during the growth, including root length, stem length, total plant weight, green tissue weight (tiller, leaf), and total chlorophyll and chlorophyll a/b ratio. The objective of this work was to investigate the growth parameters of *P. canariensis* L. when cultivated under a persistent contamination of farming fuels.

2 Materials and Methods

2.1 Plant Growth

The nutrient conditions were performed at pH 7.6 taking into account the culture conditions as previously described Putnam et al. (1990). It was achieved in 1.4 % (*w*/*v*) agar-agar with mineral salts (MS): PO4K2H (2.93 g/l), PO4H2K (0.77 g/l), NH4CL (2.25 g/l), MgSO4.7H20 (0.45 g/l), SO4Fe (0.135 g/l), and Cl2Ca (0.45 g/l). We used a modified method of Alarcón et al. (2006) and Bushnell and Haas (1941). Melted agar with MS was poured inside Petri dishes with four different commercial fuels (premium grade−99 octanes gasoline, regular grade−93 octanes gasoline, diesel fuel−51 cetanes, or kerosene BS2869 Class C2) in acetone to obtain an even distribution of fuel in the agar. Then, the acetone was evaporated off in a fume cupboard. Twenty seeds of *P. canariensis* L. previously sterilized in a solution of Triton X-100 (0.04 %*v*/*v*) and bleach (30 %*v*/*v*), were added to each plate. In order to avoid loses of volatile hydrocarbons, plates were immediately covered with a lid after seeding, and liquid fuels with the proper concentration ((*v*/*v*): 1, 3, or 10 % for each one) were layered on the surface of the solid agar. This thin layer was replenished every 7 days to keep constant the fuel concentration. Seven replications were used per treatment. Plates with acetone but without the addition of hydrocarbon were used as negative controls. The experiments were conducted in a growth chamber with a 16/8 light/dark cycle and a constant temperature of 20 °C, the best condition for an optimum growth of *P. canariensis* L. (Scurfield 1963). All the plates were watered with sterilized distilled water every week to maintain the agar moisture.

2.2 Plant Growth and Physiological Parameters

Plants were observed and monitored every 2 days. Plant samples were taken on day 60 for chlorophyll analysis (the negative effect of fuels on plants was greater during early growth). The fresh weight as well as the length of stems and roots was also measured on day 60. There were seven replications per treatment. Chlorophyll content of plants was measured according to Moran and Porath (1980). Two hundred milligrams of plant leaves were incubated in 80 % acetone for 24 h, at 4 °C, in the dark. Absorbance of the solutions was measured with a spectrophotometer (Shimadzu, UV1601PC) at 645 and 663 nm. Chlorophyll concentrations were calculated using the following equations:

$$[\text{Chl a}] = [12.7 \text{ x } A_{663}] - [2.69 \text{ x } A_{645}]$$
$$[\text{Chl b}] = [22.9 \text{ x } A_{645}] - [4.68 \text{ x } A_{663}]$$
$$[\text{Total Chl}] = [8.02 \text{ x } A_{663}] + [20.2 \text{ x } A_{645}]$$

Then chlorophyll contents were expressed based on fresh weight of plants in milligrams/grams.

2.3 Statistical Analysis

The applied statistical design was the factorial analysis, because there are two factors “type of oil” and “hydrocarbon concentration”, each with four (1: premium, 2: diesel, 3: regular, 4: kerosene) and three levels (1: 1 %, 2: 3 %, 3: 10 %), respectively.

The first stage consisted of univariate descriptive analysis of each variable: (root length, stem length, total plant weight, green tissue weight (tiller, leaf), and total chlorophyll and chlorophyll a/b ratio) (Table 1). To analyze the normality of these variables, the Shapiro Wilk test was applied.

Since the aim of the work was to analyze how each fuel and its concentration affect the growth and survival of *P. canariensis*, a factorial analysis was performed (Table 2). Subsequently, we applied the Tukey test to detect significant differences between means where variance analysis was significant.

The data analysis was done with the R Project for Statistical Computing software version 2.12.0 (www.r-project.org).

3 Results

Table 1 shows the univariate analysis of the observed variables (root and stem length, biomass, and chlorophyll content) during the growth of *P. canariensis* under different fuels at diverse concentrations. The Shapiro Wilk test indicated that all variables are normally distributed, as the *p* value yielded by the test was significantly higher than the alpha level (0.05) for all variables.

In a second step, we applied the variance factorial analysis (Table 2). The fuel factor has four levels (1: premium, 2: diesel, 3: regular, 4: kerosene), while the concentration factor has three levels (1: 1 %, 2: 3 %, 3: 10 %), whereby the interaction effect has 12 categories (4 types of fuels×3 concentrations).

Factorial analysis of variance showed that for all studied variables, the effect of concentration and types of fuel as well as the interaction between both factors (type and concentration) were significant (Table 2).

Finally, the Tukey test was applied to detect the significantly different means for each observed variable (Fig. 1). The interaction between the two factors does not exist when the lines resulting from joining the mean values obtained with each method are parallel or coincident. On the other hand, the points where the different lines are in contact represent the interaction of both factors (type and concentration of fuel) which affects the analyzed variable.

3.1 Effect of Fuels on Root Length

Table 1 described the behavior of the variable under different type and concentration of fuels, and variance factorial analysis showed (Table 2) that the four fuels at different concentration affected the root length. It is observed (Table 1) that root length at 1 and 3 % with premium fuel; 1, 3, and 10 % with regular fuel; and 1 % with kerosene are significantly longer than the control

Table 1 Univariate analysis of the observed variables during the growth of *P. canariensis* under different fuels at diverse concentrations. Variables: chlorophyll content, root and stem length, and plant and green tissue weight of *P. canariensis* L during plant exposition to different fuels. Chlorophyll contents were calculated based on fresh weight of plants in milligrams per gram (mg/g). Length and weight were calculated in centimeter (cm) and grams (gr), respectively. $\bar{x}$± *SD* , mean±standard deviation

Variables	Control group ($\bar{x}$± *SD*)	Fuels (%)	Commercial fuels			
			Premium	Diesel	Regular	Kerosene
Root length	6.75±0.50	1	10.67±0.33	6.13±0.32	10.40±0.12	9.67±0.07
		3	10.43±0.19	0.00±0.00	10.68±0.02	2.37±0.08
		10	7.97±0.45	0.00±0.00	10.23±0.21	0.00±0.00
Stem length	12.5±0.58	1	9.64±0.37	4.65±0.31	10.33±0.09	5.72±0.02
		3	9.38±0.10	0.00±0.00	10.65±0.11	1.25±0.17
		10	4.20±0.33	0.00±0.00	7.50±0.65	0.00±0.00
Plant total weight	1.20±0.08	1	0.86±0.06	0.36±0.03	0.93±0.02	0.67±0.06
		3	0.83±0.02	0.15±0.03	0.88±0.02	0.13±0.00
		10	0.63±0.03	0.13±0.01	0.81±0.05	0.13±0.00
Green tissue weight	0.43±0.01	1	0.18±0.03	0.04±0.01	0.26±0.01	0.14±0.02
		3	0.24±0.02	0.00±0.00	0.18±0.00	0.09±0.01
		10	0.13±0.02	0.00±0.00	0.16±0.01	0.00±0.00
Chlorophyll a/b ratio	4.5±0.58	1	4.33±0.14	0.95±0.07	3.67±0.07	0.66±0.14
		3	4.21±0.14	0.00±0.00	3.27±0.12	0.29±0.11
		10	3.85±0.13	0.00±0.00	2.44±0.11	0.00±0.00
Total chlorophyll content	2.75±0.96	1	2.77±0.10	0.43±0.07	2.22±0.09	0.44±0.05
		3	2.48±0.05	0.00±0.00	1.83±0.04	0.09±0.05
		10	2.22±0.03	0.00±0.00	1.57±0.14	0.00±0.00

group. We also observed extremely curled roots (Fig. 2) at these concentrations. This deformation was not seen in controls assays without fuels.

When considering the effect that the interaction between type and concentration of fuel has over the root length, we observed (Fig. 1) that the means of the root length with regular at 1, 3, or 10 %, and premium 1 or 3 % did not show significant differences. This means that the different concentrations of regular or premium fuel do not produce effects on the variable (root length). Therefore, the effect on the variable produced with premium is the same to those produced in presence of

Table 2 Variance factorial analysis. We studied the effect of concentration and type of fuel as well as the interaction between both factors, for all variables under studio (chlorophyll content; root and stem length, and plant and green tissue weight). Zero values of *P* indicate that the means of the variables under study are different under the effect of each factor (concentration and type of fuel) as well as with the interaction between both (concentration× type)

Variable	Type	HC Concentration	Type and HC concentration
Root length	0.00	0.00	0.00
Stem length	0.00	0.00	0.00
Plant weight	0.00	0.00	0.00
Green tissue weight	0.00	0.00	0.00
Chlorophyll a/b ratio	0.00	0.00	0.00
Total chlorophyll content	0.00	0.00	0.00

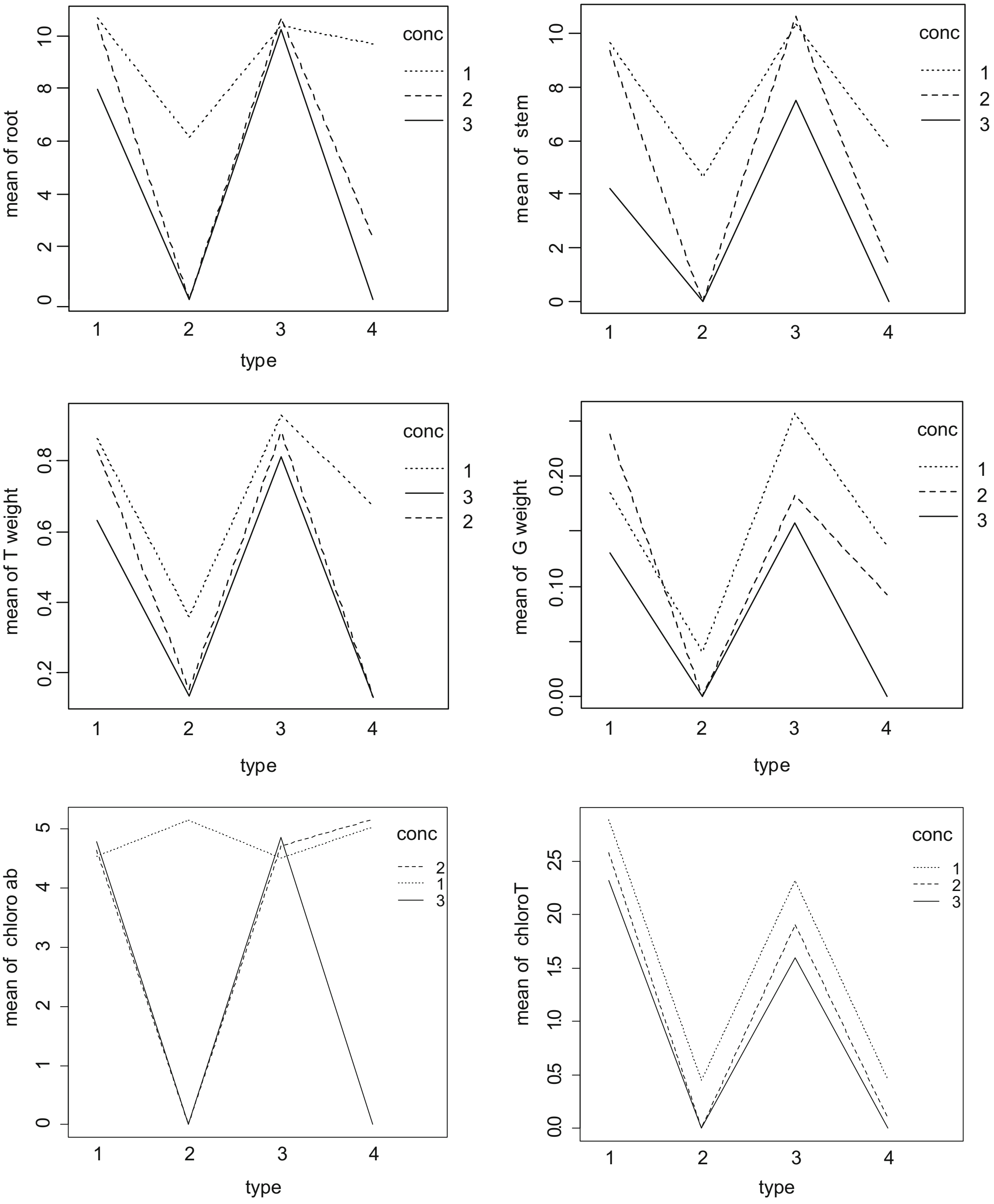

Fig. 1 Tukey results for the interaction between type and concentration of fuel over the variable. Tukey test was applied to detect the significantly different means for each observed variable: root (root length), stem (stem length), T weight (the plant total weight), G weight (the weight of the plant green tissue), Chloroab (chlorophyll a/b ratios), and chloro T (total chlorophyll content) according to the type of fuel (Premium, Diesel, Regular, Kerosene) and concentration (CONC (*v*/*v*), 1, 3, 10 %). Chlorophyll contents were calculated based on fresh weight of plants as milligrams per gram (mg/g) of fresh weight. Length and weight were expressed in centimeters (cm) and grams (gr), respectively. *Parallel lines* mean that the interaction has no significant effect, namely the interaction of the two factors do not affect the analyzed variable. Otherwise, the interaction has significant effect

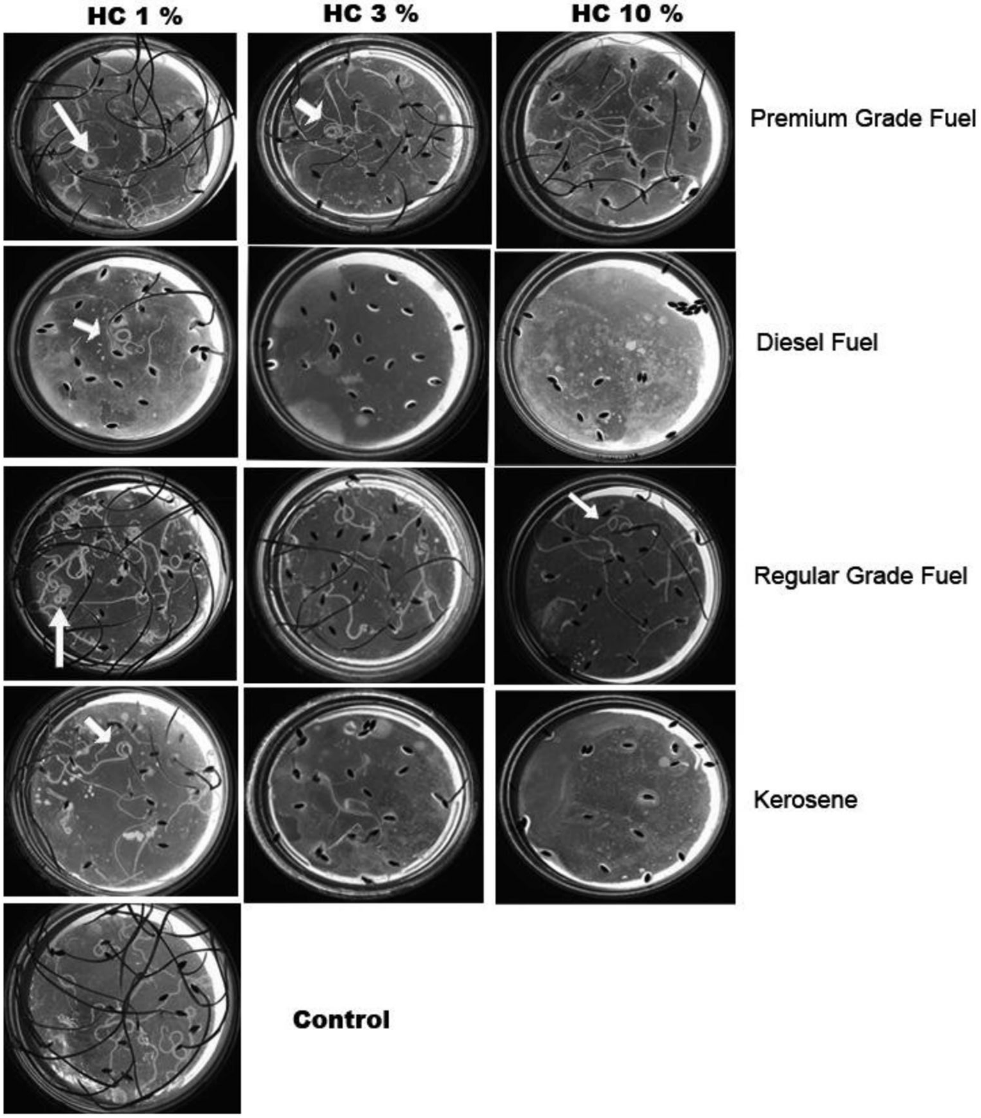

Fig. 2 Plant growth after 10 days of the experiment. *White arrow* indicates characteristic curled roots when the seeds germinated under fuels. HC 1, 3, and 10 % indicate fuel concentration (*v*/*v*). The "control" assay was prepared in agar and mineral salt but without the addition of fuels

regular. The most harmful effect on root length was produced with diesel at 3 or 10 % and kerosene 10 % with means without significant differences. The interaction effect between type and concentration of fuel is significant since the lines are not parallel.

3.2 Effect of Hydrocarbon Fuels on the Length of the Stem

After the univariate analysis of the observed variables (Table 1), variance factorial analysis showed (Table 2) that the interaction between the types of hydrocarbons and different levels of concentration affect the stem length. In particular, with the Tukey test we have seen (Fig. 1) that the means of the stem length exposed at 1 or 3 % of regular fuel were less affected and did not differ significantly between them. This means that the effect produced for the interaction will be the same.

The stem length was more affected by premium at 1 or 3 % than regular fuel at the same concentration. The most harmful effect on the length of the stem is 3 or 10 % diesel or 10 % kerosene.

The interaction effect between type and concentration of fuel is significant.

3.3 Effect of Hydrocarbon Fuels on Total Plant Weight

Table 1 described the behavior of the variable under different type and concentration of fuels. The interaction between the two factors (type and concentration of fuels) altered plant weight in all fuel types at different levels of concentration as was seen in Table 2. The Tukey test (Fig. 1) showed that regular fuel at 1 % affected less the total plant weight than regular at 3 or 10 %, and premium at 1 or 3 %. Therefore, the plant weight will be affected in the same way if the plant grows under regular at 3 or 10 %, and premium at 1 or 3 %. The most harmful effect on plant weight was produced with diesel or kerosene at 3 or 10 %, which presented means without significant differences.

The interaction effect between type and concentration of fuel is significant.

3.4 Effect on Green Tissue Weight

The behavior of the variable under different type and concentration of fuels is seen in Tables 1 and 2 showed that the effect of each factor and its interaction were significant. Figure 1 describes the results after the Tukey test application and it was seen that premium 3 % and regular 1 % where the fuels which less affected the green tissue weight, and they presented the same mean.

The effect of diesel at 3 or 10 % was the same as kerosene at 10 % since they presented means without significant differences. They showed to have the more harmful effect for the development of the green tissue in grass canary plants.

The interaction effect between type and concentration of fuel is significant.

3.5 Effect on a/b Chlorophyll Ratio

Table 1 described the behavior of the variable under different type and concentration of fuels. After the Tukey test application, it was observed (Table 2) that the interaction between the two factors (type and concentration of fuels) affected the relation of a/b chlorophyll in all types of hydrocarbons at different levels of concentration. Figure 1 showed that the effect on the relation a/b chlorophyll at 1 or 3 % of premium was less harmful than premium 10 % or regular 1 %. The major detrimental effect on the a/b chlorophyll ratio was produced with diesel at 3 or 10 %, and it was the same with kerosene at 10 %.

The interaction effect between type and concentration of fuel is significant.

3.6 Effect of Fuels on Chlorophyll Content

Univariate analysis of the observed variables (root and stem length, biomass, and chlorophyll content) during the growth of *P. canariensis* under different fuels at diverse concentrations is seen in Table 1. Variance factorial analysis showed that the interaction between the two factors (type and concentration of fuel) altered green weight in all types of hydrocarbons at different levels of concentration (Table 2). In Fig. 1, we observed that each fuel affect the total chlorophyll content with all the tested concentrations. The damaging effect was reduced in presence of premium 1 or 3 %, followed by premium 10 % and regular 1 %. The most harmful effect in the total chlorophyll content was seen with diesel 3 or 10 %, and it was the same to those produced in presence of kerosene 10 % (they have the lowest mean).

The interaction effect between type and concentration of fuel is significant.

4 Discussion

The ability of *P. canariensis* L. to proliferate in the presence of different fuels varied among the type and concentrations of fuel used. It was shown that the interaction was significant; therefore, it is complex to examine separately the effects of each treatment factor. This is not often taken into account and can lead to misinterpretation. The data suggest that certain responses might be indicative for resistance and acclimation to those fuels at different concentration in the environment. Recently, some authors (MacKinnon and Duncan 2013) have shown that volatile hydrocarbons from diesel fuel delay shoot/root emergence and have a detrimental effect on plant development, with branched cyclohexanes shown to be successful at arresting growth in certain grass species. On the other hand, the more resistant species can increase water content in shoots in presence of creosote (Huang et al. 2004). PAHs are part of the heavy fuel fraction and some authors mentioned (Nakata et al. 2011; Ma et al. 2010) that PAHs can penetrate through the cell membranes and decrease water and nutrient utilization efficiency. We found that fuels such as premium and regular showed to have less toxic effects in canary grass growth compared to diesel and kerosene. Maybe the first ones, although they are more volatile (*Topping*), have less heavy products than diesel and kerosene, and the plants could keep enough water content in the shoots.

Chlorophyll content and the chlorophyll a/b ratio are often used as indicators of stress in plants (Huang et al. 2004). These parameters are used for assessing exposure of plants to most common fuels because aromatic hydrocarbons block electron transport on photosynthesis (Marwood et al. 2001). This agrees with our observations, where chlorophyll content and chlorophyll a/b ratio were affected by the presence of different fuels and concentrations.

Roots in premium (1 and 3 %), regular (1, 3, and 10 %), and Kerosene (1 %) were significantly longer compared to the control group. Plants grown in the presence of fuel have longer roots (Huang et al. 2004), suggesting that this could be one mechanism to survive under toxic conditions. Larger and deeper root systems would increase nutrient and water uptake to maintain vigorous growth in presence of PAHs. Morphological symptoms of PAH stress were deformed trichomes and impaired root hair initiation in *Arabidopsis* sp. (Alkio et al. 2005). Ethylene, the simplest alkene, affects root growth, alters the normal geotropic response of the root, and causes epinasty in plants (curvature of the leaves by excess growth in the upper face, and other deformities) (Abeles et al. 1992).

Although, there is little information on the physiological basis of curvature induction by hydrocarbon across the root cap. Over-curled roots, especially at low fuel concentration were observed in this work. This curvature may be due to differential growth that at some point becomes stabilized and long-lasting. The formation of a curve in *Arabidopsis* induces lateral root initiation and explains left/right positioning, and the formation of a curve could potentially trigger periodic increases in auxin concentration in regions as far away as the basal meristem region (Laskowski et al. 2008). Perhaps the formation of curled roots in *P. canariensis* L. induces the formation of lateral roots to find optimal conditions for growth and protection in toxic environment.

We can clearly distinguish distinct groups in our work from the behavior of the variables according to the type of fuel to which the plants were exposed: regular and premium fuel for one side, and kerosene and diesel fuel on the other side. This can be seen in Fig. 1, and from the *p* values in Table 2. The variations in the studied parameters may change as a function of the fraction of fuel used, being the heavier fraction (kerosene and diesel) the more toxic.

Adam and Duncan (2002) found that diesel fuel played an influential role in delaying seed emergence and reducing percentage germination. It has an inhibitory effect on germination by physically impeding water and oxygen transfer between the seed and the surrounding soil environment. Recently, MacKinnon and Duncan (2013) have demonstrated that the acute phytotoxicity in germination is caused by the volatile fraction of diesel fuel. We also confirmed that germination response was affected by the concentration of the specific fuel employed (Fig. 2), although we have been focused in less studied parameters such as those related with growth.

The biomass produced during the plant growth is critical for a successful remediation; therefore, this aspect should be taken into account since canary grasses are used in hydrocarbon phytoremediation (Schnoor 1997). They provide a tremendous amount of fine roots in the surface soil which is effective at binding and transforming hydrophobic contaminants such as TPH, BTEX, and PAHs. On the other hand, even if the present research was focused for the first time in persistent contamination, it has shown that the studied fuels, their concentration, and the interaction of both factors, affect the plant weight. It would play a critical role in plant biomass accumulation and subsequent effective phytoremediation. Consequently, more care is necessary when using *P. canariensis* in fuel phytoremediation to reduce the cost and time of remediation. Our results suggest that using *P. canariensis* to remediate soils contaminated with premium or regular for above 3 % (*v*/*v*) cannot be recommended. The same conclusion applies for the case of *P. canariensis* in remediation of soils contaminated with diesel or kerosene at the studied concentrations.

Future studies are required related to plant-microbial interaction with rhizosphere when the studied fuels are present in the matrix. If plants can be successfully grown in polluted soils, then the plant-microbial interaction in the rhizosphere may provide enhanced breakdown of fuels in vegetated soils as opposed to non-vegetated soils.

5 Conclusions

P. canariensis growth parameters were affected by all concentrations of the studied fuels and by the interaction between concentration and the type of fuel. This is one of the first reported cases which describe the observed variations in growth parameters of canary grass when cultivated in presence of essentially constant contamination of farming fuels. This work highlights the importance of fully understanding the soil components interaction with plant growth variables in order to enhance remediation efforts prior to establishment of expensive field trials.

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References

Abeles, F. B., Morgan, P. W., & Saltveit, M. E., Jr. (1992). *Ethylene in plant biology* (2nd ed.). San Diego: Academic.

Adam, G., & Duncan, H. (2002). Influence of diesel fuel on seed germination. *Environmental Pollution, 120*, 363–370.

Alarcón, A., Delgadillo Martínez, J., Franco Ramírez, A., Davies, F. T., Jr., & Ferrera Cerrato, R. (2006). Influence of two polycyclic aromatic hydrocarbons on spore germination, and phytoremediation potential of *Gigaspora margarita. Review International Contaminant Ambientale, 22*, 39–47.

Alkio, M., Tabuchi, T. M., Wang, X., & Colon-Carmona, A. (2005). Stress responses to polycyclic aromatic hydrocarbons in *Arabidopsis* include growth inhibition and hypersensitive response-like symptoms. *Journal of Experimental Botany, 56*, 2983–2994.

Balwin, L. L. (1922). Modification of soil flora induced by application of crude petroleum. *Soil Science, 14*, 467–477.

Burd, G. I., Dixon, D. G., & Glick, B. G. (1998). Plant growth promoting bacteria that decease heavy metal toxicity in plants. *Applied and Environment Microbiology, 64*, 3663–3669.

Bushnell, L. D., & Haas, H. F. (1941). *Journal of Bacteriology, 41*, 653.

Concepción, J. L. (1999). *Customs, traditions and medicinal remedies in Canary Island (Spain). Volume II: healing plants*. Spain: Graficolor/Cultural Association of the Canary Islands, La Laguna (Tenerife).

Deuel, L., & Holliday, G. H. (1997). *Soil remediation for the petroleum extraction industry* (2nd ed.). Tulsa Oklahoma, USA: Penn Well Publishing Co.

De viana, M. L., Plaza, J., & Ruggieri, V. (2003). Survey generation and destiny of residues of total petroleum hydrocarbons (TPH) in the city of Salta (Argentina). *Andes, 14*, 331–340.

Glick, B. R. (1995). The enhancement of plant growth by free living bacteria. *Canadian Journal of Microbiology, 41*, 109–117.

Holliday, G. H., & Deuel, L. E. Jr. (2006). Rational methods for evaluating and designing remediation programs for agricultural soils, in: Track2 Cleanup and Technology Transfer Remediation. International conference on The Future of Agriculture: Science, Stewardship, and Sustainability. Sacramento, California, USA, pp. 158–169.

Huang, X. D., El Alawi, Y., Penrose, D. M., Glick, B. R., & Greenberg, B. M. (2004). Responses of three grass species to creosote during phytoremediation. *Environmental Pollution, 130*, 453–463.

Kirchmann, H., & Thorvaldsson, G. (2000). Challenging targets for future agriculture. *European Journal of Agronomy, 12*, 145–161.

Laskowski, M., Grieneisen, V. A., Hofhuis, H., ten Hove, C. A., Hogeweg, P., Mare, A. F. M., et al. (2008). Root system architecture from coupling cell shape to auxin transport. *PLoS Biology, 6*(12), e307. doi:10.1371/journal.pbio.0060307.

Ma, B., He, Y., Chen, H., Xu, J., & Rengel, Z. (2010). Dissipation of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere: synthesis through meta-analysis. *Environmental Pollution, 158*, 855–861.

MacKinnon, G., & Duncan, H. J. (2013). Phytotoxicity of branched cyclohexanes found in the volatile fraction of diesel fuel on germination of selected grass species. *Chemosphere, 90*, 952–957.

Marwood, C. A., Solomon, K. R., & Greenberg, B. W. (2001). Chlorophyll fuorescence as a bioindicator of effects on growth in aquatic macrophytes from mixtures of PAHs. *Environmental Toxicology and Chemistry, 20*, 890–898.

Miller, R. W., & Honarvar, S. (1975). Effects of drilling fluid-component mixtures on plants and soil, in: Environmental aspects of chemical use in well-drilling operation, Conference Proceedings, May, Houston, TX EPA 560/1-75-004. pp. 125–143.

Moran, R., & Porath, D. (1980). Chlorophyll determination in tissue using N, N-dimethylforamide. *Plant Physiology, 65*, 478–479.

Nakata, C., Qualizza, C., MacKinnon, M., & Renault, S. (2011). Growth and physiological responses of *Triticum aestivum* and *Deschampsia caespitosa* exposed to petroleum coke. *Water Air Soil Pollution, 216*, 59–72.

Oram, R. N. (2004). *Phalaris canariensis* is a domesticated form of *P. brachystachys. Genetic Resources and Crop Evolution, 51*, 259–267.

Pons Jiménez, M. (2010). *Extraction of hydrocarbons and petroleum compounds in agricultural soils of the lower Tonalá river basin*. México: MSc. Thesis. Tabasco.

Putnam, D. H., Oelke E. A., Oplinger E. S., Doll J. D., & Peters J. B. (1990). Annual Canarygrass, [Internet] Alternative Field Crops Manual, University of Wisconsin-Extension, Cooperative Extension, University of Minnesota, Center for Alternative Plant & Animal Products & the Minnesota Extension Service, Madison WI, United States, 5 pp http://www.hort.purdue.edu/newcrop/afcm/cangrass.html, Accessed Dec 2013.

Scurfield, G. (1963). The effects of temperature on the early vegetative growth of *Phalaris canariensis* L. and *P. tuberosa* L. *Australian Journal of Agricultural Research, 14*(2), 165–179. doi:10.1071/AR9630165.

Schnoor, J. L. (1997). Phytoremediation, Technology Overview Report, Ground-water remediation Technologies Analysis Center, Series E, Vol. I.

Siciliano, S. D., & Germida, V. (1997). Bacteria inoculants of forage grasses enhance degradation of 2-chlorobenzoiz acid in soil. *Environmental Toxicology and Chemistry, 16*, 1098–1104.

Yagüez, J. D. (2002). Canary Grass: A forgotten culture. National Institute of Agricultural Technology. (Alpiste: Un cultivo olvidado). Instituto Nacional de Tecnología Agropecuaria. INTA - Ministry of Agricultural Subjects. Buenos Aires.