

## EFFECT OF BIOLOGICAL SOIL CRUSTS ON THE SEED GERMINATION OF THREE PLANT SPECIES UNDER LABORATORY CONDITIONS

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**Abstract:** Semiarid grasslands in northeastern Mexico contain endemic plants and animals, and are an important refuge for resident and migratory animals. Here, as in other semiarid areas, biological soil crusts (BSC) are a key component of the ecosystem. However, findings about their effect on the germination of vascular plants are contradictory. We asked whether seed germination of some native plant species would be inhibited by the presence of BSC as it has been found in other studies; in turn, we evaluated the effect of five lichens (*Endocarpon pusillum* Hedw., *Placidium* sp., *Psora cerebriformis* W.A.Weber, *Psora decipiens* (Hedw.) Hoffm., *Xanthoparmelia chlorochroa* (Tuck.) Hale), one cyanobacteria (*Nostoc commune* Vauch.), and one hepatic (*Oxymitra* sp.), from the dominant BSC of the area on the germination percentage (germinability) and speed of germination ( $t_{50}$ ) of three native plant species: *Frankenia gypsophila* I.M.Johnst., *Muhlenbergia arenicola* Buckl., and *Sartwellia mexicana* A.Gray. Germination tests were carried out in an environmental chamber at 26 °C and at a constant humidity of 60% with 12:12 hours light and darkness. BSC did not affect germination percentage of the three evaluated plant species when compared to bare soil. Speed of germination ranged between 3.5 and 5 days and there were no differences between substrates.

**Keywords:** *Endocarpon pusillum*, northeastern Mexico, *Nostoc commune*, *Placidium*, semiarid grasslands.

**Resumen:** En los pastizales semiáridos del noreste de México se encuentran plantas y animales endémicos, y son un refugio importante para animales residentes y migratorios. Aquí, como en otras zonas semiáridas, las costras biológicas del suelo son un componente clave del ecosistema. Sin embargo, los reportes sobre su efecto en la germinación de plantas vasculares son contrastantes. En este trabajo se investiga si la germinación de algunas especies de plantas nativas sería inhibida por la presencia de costras biológicas del suelo como se ha encontrado en otros estudios, y se evalúa el efecto de cinco líquenes (*Endocarpon pusillum* Hedw., *Placidium* sp., *Psora cerebriformis* W.A.Weber, *Psora decipiens* (Hedw.) Hoffm., *Xanthoparmelia chlorochroa* (Tuck.) Hale), una cianobacteria (*Nostoc commune* Vauch.) y una hepática (*Oxymitra* sp.), que son componentes de las costras biológicas del suelo dominantes en el área. Los efectos evaluados son en el porcentaje (germinabilidad) y rapidez de germinación ( $t_{50}$ ) de tres especies de plantas nativas del área: *Frankenia gypsophila* I.M.Johnst., *Muhlenbergia arenicola* Buckl. y *Sartwellia mexicana* A.Gray. Las pruebas de germinación se realizaron en una cámara ambiental a 26 °C, humedad constante del 60%, 12:12 horas luz: oscuridad. Las costras biológicas no afectaron el porcentaje de germinación de las tres especies de plantas evaluadas comparado con el suelo desnudo. La rapidez de germinación estuvo entre 3.5 y 5 días, sin diferencias entre los sustratos.

**Palabras clave:** *Endocarpon pusillum*, noreste de México, *Nostoc commune*, pastizales semiáridos, *Placidium*.

**B**iological soil crusts (BSC) are communities of organisms that live in the soils of arid and semiarid areas, and are the main living cover in many arid areas of the world (Belnap, 2006). BSC communities consist of soil surface-dwelling cyanobacteria, green algae, microfungi, bacteria, lichens, and bryophytes (Hawkes, 2003; Zhang, 2005; López-Cortés *et al.*, 2010) that grow on top or within the top layers of soil (Belnap *et al.*, 2001; Quiñones-Vera *et al.*,

2009). They influence local hydrological cycles, including soil porosity, roughness, aggregate stability, texture, pore formation, and water retention (Belnap, 2006), contribute to soil retention (Belnap and Gardner, 1993; Zhang *et al.*, 2006), and facilitate the accumulation of soil nutrients (Jafari *et al.*, 2004). Due to the effects of microtopography, and water and nutrients fluxes, BSC affect the distribution of resources in the soil, contributing to vegetation cluster patterns

(Deines *et al.*, 2007) and probably improve water infiltration and retention in the soil (Casenave and Valentin, 1989; Montaña *et al.*, 1995; Rosentreter and Belnap, 2001; Reyes-Gómez *et al.*, 2007; Yair, 2008; Chamizo *et al.*, 2010).

BSC can also affect the establishment and survival of vascular plants, and available data show varied results for BSC. Studies have shown BSC having either facilitative, inhibitory or no effect on vascular plant establishment and cover (During and Van Tooren, 1990; Belnap *et al.*, 2001). Existing evidence suggests that the effects of crusts on seed germination vary depending on the soil crust composition and plant characteristics (Lesica and Shelly, 1992; Zaady *et al.*, 1997; Prasse and Bornkamm, 2000; Rivera-Aguilar *et al.*, 2005; Serpe *et al.*, 2006; Deines *et al.*, 2007), as seeds vary in their germination requirements and consequently may respond differently to the conditions created by the crust (Baskin and Baskin, 1998). Positive effects of the crust on vascular plants may be the result of improved soil moisture conditions and nutrient availability, as well as a decrease in predation (During and Van Tooren, 1990; Belnap and Harper, 1995). Negative effects could be attributed to competition for water and nitrate, changes in the red/far-red ratio or the presence of inhibitory compounds (During and Van Tooren, 1990; Callaway and Walker, 1997).

Speed of germination results for different BSC and plant species have also been controversial. For example, Godínez-Alvarez *et al.* (2012) found no differences in  $t_{50}$  for *Agave marmorata* Roehl. and *Neobuxbaumia tetetzo* (F.A.C. Weber) Backeb. germinated on a cyanobacteria crust and on bare soil. Deines *et al.* (2007) compared germination of two grass species (*Bromus tectorum* L. and *Vulpia microstachys* (Nutt.) Benth.) on three different substrates (lichen crust, mixed crust, and bare soil) and found a longer germination time on the lichen crust than on bare soil and no differences between the mixed crust and bare soil for both grass species.

Recent studies have demonstrated that BSC dominated by *Diploschistes* sp. reduced plant emergence (Escudero *et al.*, 2007; Serpe *et al.*, 2008). This might be due to the sealing of the soil pores by these crusts that might reduce water availability and thus inhibit germination (Eldridge *et al.*, 2010). Similarly, Prasse and Bornkamm (2000) in the Negev Desert in Israel and Li *et al.* (2005) in the Tengger Desert of China found greater seedling emergence after removal or destruction of the BSC. St. Clair *et al.* (1984) observed significantly higher germination for three grass species on algal crusts compared with mixed crusts of lichens, mosses and algae. Zamfir (2000) showed that seedling emergence was much lower on lichen mats compared with moss mats for three of the four species studied.

These uneven results highlight the relevance of evaluating more specific associations of BSC and vascular plant species in order to assess seed germination, seedling emergence, and plant establishment. Hence, in this study we

determined the effect of seven dominant BSC from the Northern Mexican Plateau (Molina-Guerra, 2013) on the germination percentage and speed of germination ( $t_{50}$ ) of three native plants. While the physical barrier of crusts is likely to affect soil moisture and several stages of seedling growth, in here we asked whether germination would be inhibited by the presence of lichens as it has been found in other studies (Deines *et al.*, 2007).

## Material and methods

**Study area.** The BSC were collected in the states of Nuevo León, Coahuila, and San Luis Potosí within the Southern part of the Chihuahuan Desert in northeastern Mexico. The collection area was within 25° 13' 51" N and 101° 17' 28" W. Mean annual temperature for the region is 17.2 °C, with a minimum of -1.8 °C in January and maximum of 35.1 °C in May. Average annual rainfall is 386.43 mm, with March being the driest month (8.43 mm) and August being the wettest (58.06 mm; SMN, 2000).

According to the Lof P. and van Baren (1987; FAO-UNESCO classification), soils in the area are mainly solonchack and calcaric phaeozem, and smaller areas of chromic vertisol and luvisc chernozem (INEGI, 1981a, b; 2002). These soils have been classified into three broad groups: (1) those with a high content of calcium carbonate and no gypsum; (2) soils with similar contents of gypsum and calcium carbonate; and (3) those with high gypsum content and very little or no calcium carbonate (Pando *et al.*, 2013). Sampling areas are between 1,800 and 2,000 m altitude.

The vegetation is mainly made from communities of halophytic/gypsophyllus grasslands associated with microphyll and rosetophyll desert scrub (Estrada-Castillón *et al.*, 2010), where the most abundant plant families are: Poaceae, Chenopodiaceae, and Frankeniaceae (Rzedowski, 1978). These grasslands contain several endemic plants (*Dalea gypsophila*, *D. radicans*, *Frankenia gypsophila*, *Machaeranthera heterophylla*, and *M. crutchfi eldii*; Estrada-Castillón *et al.*, 2010), and animals (*Cynomys mexicanus*, *Spizella wortheni*; Baillie and Groombridge, 1996).

**Field sampling.** The dominant BSC were collected with a cylinder designed by the authors. The size of the cylinder was adjusted to the size of the Petri dishes where the samples were placed (100 × 15 mm). All Petri dishes were covered with mixed crust where at least 50% was occupied by the treatment species. Samples to be collected were previously sprayed with distilled water to facilitate their extraction. The cylinder was placed on top of the soil and pressed until the lower part of the cylinder touched the soil crust. Once the crust was extracted it was placed on a Petri dish and sealed with tape. The components of the collected mixed crusts were identified by experts from two mycology laboratories, there were: five lichens *Endocarpon pusillum*, *Verrucaria-*

ceae; *Placidium* sp., Verrucariaceae; *Psora cerebriformis*, Psoraceae; *Psora decipiens*, Psoraceae; and *Xanthoparmelia chlorochroa*, Parmeliaceae (all squamulose except *X. chlorochroa* which is usually foliose); one cyanobacteria: *Nostoc commune*, Nostocaceae; and one hepatic: *Oxymitra* sp., Oxymitraceae. Additionally, samples of bare soil from the three broad groups of soils in the region were collected to be used as controls.

Three native plant species that co-exist with the BSC were also selected and their seeds obtained: *Frankenia gypsophila* I.M.Johnst., Frankeniaceae; *Muhlenbergia arenicola* Buckl., Poaceae; and *Sartwellia mexicana* A.Gray, Asteraceae. Seeds of the first two plant species have been found to have a high germination percentage (> 80%) under laboratory conditions, using agar as a medium (Contreras *et al.*, 2012). The seeds were collected in September 2011 from plants growing within the study region; each seed lot contained a minimum of 50 individuals. The seeds were manually extracted, cleaned and selected using a stereoscopic microscope. They were then stored in plastic containers at room temperature.

There were no pre-treatments for either the seeds or the BSC before germination tests. Germination tests were carried out in an environmental chamber (Lumistell<sup>MR</sup> ICP-54) at 26 °C (following Contreras *et al.*, 2012) and at a constant humidity of 60% with 12:12 h light and darkness. The seeds were checked every 24 h (Baskin and Baskin, 1998) over one month. Light intervals were defined to resemble average day and night number of hours for these latitudes. Temperature was established according to Contreras *et al.* (2012), who worked on germination of these plant species even though her work did not involve soil biological crusts.

*Experiment set up and analysis.* There were 30 treatments arranged randomly in the germination chamber (ten substrates (BSC or soil) × three plant species) with five replicates. The substrates were: S1: soil with gypsum and calcium carbonate (Sgc) (45% gypsum and 35% calcium carbonate); S2: *Psora cerebriformis*; S3: *Placidium* sp.; S4: *Endocarpus pusillum*; S5: *Nostoc commune*; S6: soil with calcium carbonate and no gypsum (Sc) (0% gypsum and 62% calcium carbonate); S7: soil with gypsum and no calcium carbonate (Sg) (50% gypsum and 5% calcium carbonate); S8: *Xanthoparmelia chlorochroa*; S9: *Psora decipiens*; and S10: *Oxymitra* sp. Seeds were from three plant species: *Frankenia gypsophila*, *Muhlenbergia arenicola*, and *Sartwellia mexicana*. The substrates S2-S5, as well as S8-S10 were labeled with the name of the species occupying at least 50% of the Petri dish.

Ten seeds of each plant species were sown on the surface of the dominant BSC in each Petri dish (50 seeds per treatment). The seeds were sown evenly spaced on the surface of the selected species of BSC, except for those on bare soil treatment. For samples not covering 100% of the surface,

seeds were specifically placed on top of the particular soil crust component. Germination was recorded when the seed radicle emerged and was visible (Jurado and Westoby, 1992; Welbaum *et al.*, 1998). From these observations we obtained: (1) germinability, calculated as the percentage of germinated seeds at the end of the experiment (Flores and Jurado, 1998; Flores and Briones, 2001); and (2) Speed of germination or half-time of germination ( $t_{50}$ ), calculated as the time (in days) at which 50% of the seeds germinated (Grime *et al.*, 1981; Thompson and Grime, 1983; Jurado and Westoby, 1992; Flores and Jurado, 1998; Flores and Briones, 2001; Pérez-Sánchez *et al.*, 2011).

Variance was tested for normality and homogeneity and then ANOVA tests were performed. Mean comparisons were carried out using Tukey test ( $\alpha = 0.05$ ) for *Muhlenbergia arenicola* that showed homogeneity of variances ( $W = 0.553$ ) and Games-Howell ( $\alpha = 0.05$ ) for *Frankenia gypsophila* that did not have homogeneous variances ( $W < 0.0001$ ). Games-Howell test does better than the Tukey HSD if variances are unequal or if the number of replicates is fewer than five (Sileshi, 2012). All analysis were performed using SPSS statistics package ver. 19 (MX19000242) looking for differences in germination percentage between substrates for each plant species.

Speed of germination or half-time of germination ( $t_{50}$ ) was also analyzed and species were classified according to Jurado and Westoby (1992) into three categories: (1) fast (50% germination in 1-4 days), (2) moderate (50% germination in 4-7 days), and (3) slow (50% germination in 7-9 days or longer). Differences in  $t_{50}$  were analyzed by ANOVA and Tukey test, and included only those Petri dishes where at least one seed had germinated.

## Results

*Germination percentage.* As a whole, there were no significant differences in germinability between substrates ( $F = 1.09$ ,  $df = 9$ ,  $P = 0.368$ ). Germinability differed between substrates for *Frankenia gypsophila* ( $F = 3.712$ ,  $df = 49$ ,  $P = 0.002$ ) and *Muhlenbergia arenicola* ( $F = 3.094$ ,  $df = 49$ ,  $P = 0.007$ ), and there were no differences for *Sartwellia mexicana* ( $F = 1.650$ ,  $df = 49$ ,  $P = 1.34$ ). *Frankenia gypsophila* germinability was similar in all but one substrate: *Nostoc commune* where germinability was lower than in the others (66%; Table 1). The lowest germinability of *Muhlenbergia arenicola* was on bare soil with gypsum (18%), there were no differences between the other substrates.

*Frankenia gypsophila* showed the highest germination percentage ( $91.4\% \pm 13$  sd), followed by *Muhlenbergia arenicola* ( $56.6\% \pm 17$  sd) and *Sartwellia mexicana* ( $13\% \pm 9$  sd). This ranking was consistent when evaluating germination percentage by substrate, except for *Nostoc commune* where *M. arenicola* had a higher germination percentage (78%) than *F. gypsophila* (66%).

*Speed of germination or half-time of germination ( $t_{50}$ ).* The  $t_{50}$  was analyzed only for those seed species whose seed germination percentage was above 15% in all substrates. This threshold was established because germination percentages below 15% could generate  $t_{50}$  values that did not represent the species speed of germination, but rather reflected that those seeds might require other treatments to promote germination. Hence,  $t_{50}$  is not shown for *Sartwellia mexicana* seeds.

*Frankenia gypsophila* and *Muhlenbergia arenicola* seed germination started on the second day after sowing and had an average  $t_{50}$  of 4.86 and 3.65 days, respectively. According to Jurado and Westoby (1992), germination for *M. arenicola* was fast, and moderate for *F. gypsophila* across substrates. Speed of germination ( $t_{50}$ ) was equal for all substrates ( $F = 1.66$ ,  $df = 9$ ,  $P = 0.109$ ) and their values ranged between 3.5 and 5 days (Table 1).

## Discussion

*Germination percentage or germinability.* Results from this research indicated that, under laboratory conditions, the BSC did not affect the seed germination percentage of the three evaluated plant species from the Northern Mexican Plateau.

In agreement with this study's findings, Li *et al.* (2005) found no differences in germination percentage and seedling establishment between soils with biological crusts and bare soil for *Eragrostis poaeoides* Beauv. and *Bassia dasyphylla* O.Kuntze under wet conditions in the Tengger Desert in Northern China. Deines *et al.* (2007) found negative effects for BSC on the germination of *Bromus tectorum* L. and

*Vulpia microstachys* Nutt., where germination percentage, analyzed under laboratory conditions, was about three times lower on the BSC dominated by the lichen *Diploschistes muscorum* (Scop.) R.Sant, than on bare soil. Germination on bare soil was found to be equal to mixed biological crusts which contain the lichens *Aspicilia*, *Caloplaca*, *Candelariella*, *Collema*, and *Placidium*, the cyanobacterium *Microcoleus*, and a low growing form of the moss *Syntrichia caninervis* Mitt. Zaady *et al.* (1997) found that germination of three mucilaginous seeds decreased on soils with a smooth crust dominated by cyanobacteria.

The effects of the BSC on germination may vary according to plant species, the type of biological crust (either smooth or rough), and the species that dominate in the crust (Rivera-Aguilar *et al.*, 2005; Deines *et al.*, 2007), as well as the size and morphology of the seeds and the presence of microsites in the crust suitable for germination (Li *et al.*, 2005). Escudero *et al.* (2007) and Serpe *et al.* (2008) showed that BSC reduced seedling emergence when it was dominated by species like *Diploschistes* sp., because this lichen might seal the soil, thus reducing water availability during the germination period of some plants (Otsus and Zoel, 2004; Morgan, 2006). Contrastingly, the presence of both algal and moss crusts significantly enhanced the germination of *Bassia dasyphylla* (Fisch. & Mey.) O.Kuntze and *Artemisia ordosica* Krasch. compared with uncrusted soil (Su *et al.*, 2009).

Godínez-Alvarez *et al.* (2012) found that biological soil crusts increased germination of one species (*Agave marmorata*) but did not show any effect on the germination of two other species from the same region (*Prosopis laevigata* (H. & B.) Jonhst. and *Neobuxbaumia tetetzo*). Germination of

**Table 1.** Germination percentage and speed of germination ( $t_{50}$ ) (average and standard error,  $n = 5$ ) per plant species by substrate.

Substrate	<i>Frankenia gypsophila</i>		<i>Muhlenbergia arenicola</i>		<i>Sartwellia mexicana</i>		$t_{50}$	
	%	S.E.	%	S.E.	%	S.E.	%	S.E.
Bare soil with gypsum and calcium carbonate	100	0.00	60	7.07	10	5.47	56.7	4.0
<i>Psora cerebriformis</i> W.A.Weber	90	4.47	48	11.14	10	3.16	49.3	4.7
<i>Placidium</i> sp.	98	2.00	52	11.58	0	0.00	50.0	3.6
<i>Endocarpom pussillum</i> Hedwig	100	0.00	70	3.16	2	2.00	57.3	3.5
<i>Nostoc commune</i> Vauch.	66	11.66	78	10.20	14	5.10	52.7	4.9
Bare soil with gypsum	96	4.00	18	5.83	30	16.43	48.0	4.7
<i>Xanthoparmelia chlorochroa</i> (Tuck.) Hale	70	15.81	54	9.27	20	5.48	48.0	5.0
<i>Oxymitra</i> sp.	96	2.45	70	8.94	14	5.10	60.0	4.1
Bare soil with calcium carbonate	100	0.00	54	14.35	12	5.83	55.3	3.6
<i>Psora decipiens</i> (Hedwig) Hoffm.	98	2.00	62	7.35	18	4.90	59.3	4.1



*A. marmorata* was significantly higher on a cyanobacteria crust (72.5%) than on a mixed crust (30%) that contained cyanobacteria and moss (mainly *Bryum* and *Pseudocrossidium* species). Germination on bare soil (52.5%) had intermediate values that did not differ significantly from any of the BSC treatments. Godínez-Alvarez *et al.* experiment was also conducted in a laboratory and at constant humidity for all treatments. Zhang *et al.* (2010) found contrasting results using moist and dry conditions for five species in the China Gurbantunggut Desert, the presence of BSC resulted in less seeds germinating in two out of five species under dry conditions, and in three out of five species under moist conditions.

*Speed of germination ( $t_{50}$ ).* Seeds of *Muhlenbergia arenicola* and *Frankenia gypsophila* showed average  $t_{50}$  of 3.65 days (fast) and 4.86 days (moderate), respectively. These short germination times could reflect the response of the seeds to one single event of profuse rainfall, typical for an environment where wet soils are uncommon and humid periods are brief (Jurado and Westoby, 1992), which are typical climatic characteristics for the area where the seeds chosen for this study came from.

Speed of germination was similar across substrates. Likewise, Godínez-Alvarez *et al.* (2012) found that the number of days to 50% of the total germination ( $t_{50}$ ) for *Agave marmorata* and *Neobuxbaumia tetetzo*, from semiarid Valle de Tehuacan in Mexico, were between 1.88 and 5.5 days for the different species and treatments with no statistical differences between them. In contrast, in the same area of Valle de Tehuacan in Mexico, Rivera-Aguilar *et al.* (2005) evaluated seed germination rate using quadratic regressions for two native plant species (*Mimosa luisana* Brandege and *Myrtillocactus geometrizans* (Martius) Console) concluded that seeds placed in BSC had a faster germination rate compared to those placed in bare soil. Contrarily, Deines *et al.* (2007) found that mean germination time (MGT) of *Vulpia microstachys* Nutt. and *Bromus tectorum* L. was faster on mixed crusts and on bare soil than on crusts of lichens, with an average of 5-7 days for the first two conditions, and 11 days for the crust of lichens. Also working with grasses (*Festuca idahoensis* Elmer, *Festuca ovina* L., *Elymus wawawaiensis* J. Carlson & Barkworth, and *Bromus tectorum* L.), Serpe *et al.* (2006) found that MGT was shorter for seeds germinating on bare soil than on BSC made of short or large mosses (*Tortula ruralis* Hedw. and *Bryum argenteum* Hedw.), and Briggs and Morgan (2011) found that two out (*Salvia verbenaca* and *Vittadinia gracilis*) of the five species tested, exhibited shorter  $t_{50}$  on disturbed soil crust than on moss and foliose lichen. The other species tested were *Austrodanthonia* sp. (Poaceae), *Maireana excavata* (Chenopodiaceae), and *Leptorhynchus scaber* (Asteraceae), all common in the semi-arid woodlands to the northwest of Melbourne, Australia.

These somehow uneven results on speed of germination ( $t_{50}$ ) seem to be a consequence of differences on the way the experiments were carried out (e.g. providing or not constant humidity), as well as the roughness of the evaluated BSC and the dominant species on the crust.

Further experimental and field evidence is required to better understand the implications of BSC on seed germination and seedling establishment.

## Conclusions

The purpose of this paper was to assess whether the more conspicuous types of BSC found in the semiarid grasslands of northeastern Mexico inhibit or delay seed germination of vascular plants as has been reported for other biological soil crusts. By analyzing the effect of five lichens, one cyanobacteria, and one hepatic on the germination of native plants, we conclude that under high substrate humidity conditions, BSC did not inhibit or delay seed germination of the vascular plants used for this study.

*Muhlenbergia arenicola* and *Frankenia gypsophila* had fast and moderate average speed of germination or half-time of germination ( $t_{50}$ ) of 3.65 days and 4.86 days, respectively, according to the expected germination times in environments where there are only brief periods of high soil humidity. Values for  $t_{50}$  were between 3.5 and 5 days and did not differ between substrates.

## Acknowledgements

This work was funded by the PRACTICE (Grant No. 226818) and UANL PAICYT (No. CN-064-09) projects.

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Received: April 8th, 2013

Accepted: July 7th, 2013