

Competitiveness of generalist plant populations at a mine site

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Abstract: Plants inhabiting extremely stressful mine site environments tend to be specialized and localized, where they express lower performance than nonmine site plants from adjacent areas. However, such a cost may be concealed. In a previous study at a mine site, we found mine and adjacent nonmine plants of multiple species expressed similar performances in the absence of competition. However, a lower competitive ability may be a concealed driver. We aim to test whether costs under competition could explain specialization. In a glasshouse experiment, we measured the performance (i.e. vegetative and reproductive growth) of mine and nonmine potted plants under competition. The 6 herbaceous species tested were *Anagallis arvensis* L., *Cirsium vulgare* (Savi) Ten., *Conyza sumatrensis* Retz., *Echium vulgare* L., *Oxalis chnoodes* Lourteig, and *Senecio diaschides* D.G.Drury. We exposed individuals to interspecific competition using a local grass (*Polyopogon monspeliensis*), as well as to intraspecific competition. Plants were grown alone for the control group. For all treatments, the mine plants expressed similar performances to the nonmine plants, except for mine site *O. chnoodes*, which had lower performance under intraspecific competition. Mine plants of *A. arvensis* and *C. sumatrensis* had higher performance than nonmine plants. Overall, there was no evidence of specialization in the mine site plants. These results indicate that, under some circumstances, inhabiting a stressful metal habitat does not promote specialization in multiple species. Future research may focus on assessing the environmental conditions and population genetics that promote the evolution of generalists that inhabit extremely stressful environments.

Key words: Mine site, abiotic stress, specialization, trade-off, performance

1. Introduction

Selection in extremely stressful habitats can promote the evolution of specialized genotypes (Kawecki and Ebert, 2004). Performance trade-offs across habitats can limit the potential for genotypes to colonize all encountered environments, where locally adapted populations inhabiting stressful areas perform well in their habitat of origin but poorly in other habitats. Genotypes from stressful areas tend to have poorer performance compared to adjacent genotypes (e.g., nonspecialized ancestral genotypes) (Antonovics and Bradshaw, 1970; Agra et al., 2010). Therefore, genotypes specialized to extreme environments are predicted to be restricted to their home environments. The presence of heavy metals can impose strong selective forces that lead to specialization to local conditions, including the evolution of metal tolerance (Macnair, 1987; Wilson, 1988). Similarly, plants specialized to heavy metals may be restricted to their habitats and indicate the presence of local adaptation (Posthuma and van Straalen, 1993; Shu et al., 2002).

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Plants cope with heavy metals via the production of metal chelates and the maintenance of redox reaction mechanisms at the cellular level (Viehweger, 2014). Metal tolerance mutations may also be associated with specialization (Agra et al., 2010). However, evidence of specialization may not always be detected (Dyer et al., 1993; Marambe and Amarasinghe, 2002; Dechamps et al., 2008; Fazlioglu et al., 2017; Wan et al., 2017). Genotypes inhabiting stressful areas can express equal performances to those of adjacent genotypes, regardless of the presence of stress (i.e. master-of-all genotypes) (Remold, 2012). For example, pseudometallophyte and metal hyperaccumulator plant species can easily adapt and readapt to mine habitats (Meyer et al., 2010) and demonstrate relative indifference to elevated versus background metal levels (Baker and Proctor, 1990).

Ecological specialization may be associated with costs in the presence of enemies (e.g., herbivores or competition) (Vila-Aiub et al., 2009). Stressful habitat specialists tend to

express lower performance under competition compared to nonspecialized ancestral genotypes (Warwick and Black, 1981; Ahrens and Stoller, 1983; Gassman and Futuyama, 2005; Wan et al., 2016) because they may invest their resources in stress resistance, which trade off with performance traits, such as biomass (Grubb, 2016). Therefore, poor performance under competition could explain the restriction of specialists to their habitats, particularly in cases where there is a lack of clear costs under low-stress conditions (Harper et al., 1997; Wan et al., 2017). It is currently unknown why some stress habitat specialists remain restricted despite expressing higher performance than adjacent area specialists (Délye et al., 2013; Wan et al., 2017). Competition with unspecialized conspecifics in low-stress surroundings can limit the spread of specialists away from their habitats (Cook et al., 1972; Hickey and McNeilly, 1975; Gomulkiewicz and Houle, 2009). Trade-offs may not be apparent when plants are grown alone but can be detected under conspecific competition (Cook et al., 1972).

For plants in stressful habitats, a lower performance in the presence of competitors may be a key principle in promoting specialization to extremely stressful habitats. For example, Hickey and McNeilly (1974) tested multiple herbaceous species at a mine site and found that mine plants tended to have lower fitness under competition than nonmine plants. Similarly, another study on herbaceous metal-tolerant plants also found lower competitive ability in mine plants when exposed to competition from nonmine plants (Cook et al., 1972). There were also no differences among the biomass of mine and nonmine plants when grown in pure stands without intraspecific competition (Cook et al., 1972). Similarly, assessments of the competitive ability of herbicide-tolerant and adjacent nontolerant invasive plants have also demonstrated a competitive cost to specialization (e.g., Holt, 1988; Williams et al., 1995; Vila-Aiub et al., 2009). Thus a competitive cost may play a role in the divergence of the two populations.

There is a lack of studies testing the competitive ability of mine and adjacent nonmine plants within species, even though it is generally assumed that mine plants are less competitive than nonmine plants (Dechamps et al., 2011). We previously carried out a study on multiple herbaceous species at a mine site and the adjacent area where we found that mine and nonmine plants of all the species had similar performances in the absence of competition (Fazlioglu et al., 2017). Here we aim to test mine plants for a competitive cost of specialization by exposing both mine and nonmine plants of multiple species to competition as well as intraspecific competition. We tested the prediction that mine site populations will be more negatively affected

by competition than adjacent area populations, even where the growth and reproductive performance of mine site plants are equal or greater than adjacent area plants in the absence of competition (e.g., Cook et al., 1972).

2. Materials and methods

2.1. Study site and species

The abandoned silver mine (Sunny Corner) is located at the end of a small valley that extends downhill in a northerly direction, with a run-off creek running down the valley. The study area is located near Lithgow, New South Wales, Australia (33°22'28.85''S, 149°53'35.85''E). The mine started operations as a gold mine in 1865, and silver mining began around 1877 (Argent Minerals Ltd. website, accessed Nov 2018); the mine was abandoned by 1922 (Hayes et al., 2003). Soil pH at the mine site was very low (pH 3.5) in comparison with an uncontaminated reference site (pH 7.9). Just prior to the study, measurements of soil pH recorded using a pH soil meter (Lutron PH-220S at 22 °C) indicated that the pH inside the mine site was between 2.5 and 3. Up to 34% of toxic soil metals (i.e. copper and zinc) were in biologically available forms, and high levels of dissolved toxic metals were recorded in the mine waters and in the run-off creek (Hayes et al., 2003). The levels of zinc, lead, and copper in the mine spoils were estimated to be 3.7%, 2.1%, and 0.39% by volume, respectively (Argent Minerals Ltd. website, accessed Nov 2018). A previous survey of the adjacent area and the creek embankments found relatively low levels of soil metal; thus the contamination was concentrated inside the mine site and in the run-off into the valley stream only (Hayes et al., 2003). The soil in the surrounding area consists of loam and clay loam with angular, fine gravel (2–6 mm), and gravel (6–20 mm; i.e. Krasnozem (GSG), Gn4.11 (PPF)) (Evans Shire survey, Soil Profile Report OEH, accessed Nov 2018).

In autumn, all the herbaceous plant species present at the mine spoils were examined for seeds. Any available seeds were collected in paper envelopes (late March 2014). Then the corresponding plants outside the mine spoils were also examined for seeds. Seeds were collected from a total of 5–18 individuals per species for both the mine site and adjacent nonmine areas, with the number of individuals depending on the number of plants or seeds available within each area. The seeds were then taken to the University of New South Wales, Australia (UNSW), where they were stored in a cold room at 4 °C in the dark. The plant species that were included in the experiment were dependent on success in germinating the seeds (more details on the experimental design below).

We used a total of six short-lived herbaceous species, namely *Senecio diaschides* D.G.Drury (Asteraceae),

Conyza sumatrensis Retz. (Asteraceae), *Anagallis arvensis* L. (Primulaceae), *Echium vulgare* L. (Boraginaceae), *Oxalis chnoodes* Lourteig (Oxalidaceae), and *Cirsium vulgare* (Savi) Ten. (Asteraceae). Seeds of *Geranium solanderi*, *Poa meionectes*, and *Verbascum virgatum* were also collected, but these species produced too few viable seedlings in the glasshouse (i.e. not enough replicates) and thus were excluded. Henceforth, these six study species will be referred to by their generic names only for simplicity. Other herbaceous species present at the site include *Plantago lanceolata*, *Hypochaeris radicata*, and *Trifolium repens*, whose individuals were very small in size, not reproducing at the time of sampling, and were much less abundant. Therefore, these species could not be included in the experiment. The herbaceous species present inside the mine site represent a subset of a larger number of species inhabiting the adjacent area located approximately 1.1 km away from the mine site.

Senecio and *Oxalis* are native to Australia. In contrast, *Echium*, *Cirsium*, *Conyza*, and *Anagallis* are invasive species and they were introduced to Australia approximately 200 years ago. Herbarium data indicate all invasive species had a point of introduction in Sydney with the exception of *Echium* in Bathurst (a town inland around 25 km west of the mine site). The area around the mine site was colonized by all invasive species between 1960 and 1980 (The Atlas of Living Australia, <http://www.ala.org.au>, and PlantNET, <http://plantnet.rbgsyd.nsw.gov.au>). *Senecio* and *Conyza* have erect growth forms with terminal inflorescences. *Echium* and *Cirsium* have mostly basal growth forms and produce inflorescences terminally on upright elongated stems. *Anagallis* and *Oxalis* have a spreading habit.

2.2. Experimental design and treatments

In spring (July 2014), all seeds were taken out of cold storage. Seeds of each species from each location were pooled and sown in separate germination flats containing standard potting mix (composition: "Australian Native Landscape - Organic Garden Mix" containing 50% black soil, 20% coarse sand, and 30% composted organics). The plants were grown in a temperature-controlled glasshouse located at UNSW (temperature range: 17–27 °C, humidity: 65%), and were watered via an automatic sprinkling system two times a day.

After 5 weeks of growth (in mid-August), the seedlings with two to four true leaves were randomly chosen and carefully transplanted into individual pots (10 cm diameter × 9 cm depth) containing a growth medium (1:1 volume ratio coco-peat to river sand) with amended nutrients (Osmocote, Scotts Australia: N, P, K ratio: 19:2:5 at 1.66 g/L). The seedlings were randomly assigned to the control, low, and high competition treatment groups. A 'no competition' control group was also included where

the plant was grown alone. The plants were arranged in complete random blocks consisting of one individual from each unique species × location × competition combination (7 blocks with 36 plants per block). In addition, a week before the transplant, seeds of the competitor *Polypogon monspeliensis* were sown in a separate germination flat. For the low and high competition treatments, three individuals of the grass competitor were planted around the subject plant during the time of transplant (i.e. mid-August). The low competition treatment consisted of clipping the grass back to 10-cm tall weekly. The high competition treatment consisted of the grass competitor being left to grow. In a previous experiment involving the same size of pots used in this experiment, clipping the grass competitor to 5 cm was adequate for simulating low levels of competition, whereas unclipped grass would grow extensively in the pots and is appropriate as a high competition treatment (Wan et al., 2016). *P. monspeliensis* is an introduced grass species widespread throughout Australia and overlaps the ranges of all study species (PlantNET, <http://www.plantnet.rbgsyd.nsw.gov.au>). This species is common in the study area, although not present inside the mine site. There were seven replicates within each treatment group. Additionally, at the time of transplant, an intraspecific competition experiment was established where a mine plant was planted with a nonmine plant in the same pot. Within each pot, an individual from the mine site was planted next to a nonmine plant, equidistance from each other and to the side of the pot. There were insufficient *Cirsium* seedlings available for the intraspecific competition trial. There were seven replicates per species. The whole experiment was concluded in early March 2015, around 5 months after the start of the experiment (i.e. after the transplanting). *Echium* and *Cirsium* did not flower by the end of the experiment, whereas *Anagallis*, *Oxalis*, *Conyza*, and *Senecio* had finished flowering.

2.3. Measurement of performance

We measured plant performance as the total vegetative and reproductive output. For each plant, the number of leaves was measured as the highest number of leaves attained. Reproductive output was measured as the number of flowers produced by the plant. In addition, the timing of flowering (time at the appearance of the first bud), total dry weight, and specific leaf area (SLA) were also measured. SLA (leaf area/dry mass) was assessed to examine plant allocation to rapid growth versus longevity. SLA is a life-history trait that indicates how much resource plants are allocating to growth over time and is positively correlated with faster growth rates and phenology (Wilson et al., 1999) (i.e. high SLA is related to fast growth and reproduction; low SLA is related to resource conservation and slow growth). Assessment of the allocation or life-

history strategy used by plants under competition could be useful in interpreting plant responses (Jiménez-Ambriz et al., 2007; Wan et al., 2016). At the end of the experiment, all plant materials including the roots were cleaned, and then dried in a drying oven at 60 °C for 24 h. The material was then weighed to measure the total dry weight (g). For SLA, three fully expanded nonsenescent leaves from each plant were randomly picked. Leaf area was scanned and measured using Leaf Area Measurement software (ver. 1.3, University of Sheffield, UK). The mean SLA (mm²/mg) was recorded.

2.4. Statistical analyses

For each species, two-way ANOVAs were used to analyze location (mine site and nonmine adjacent area), competition (control, low, and high), and the location × competition interaction effects on the variation in leaf number, flower number, and SLA. Block was included as a random effect in the analyses. Tukey's honest significant difference (HSD) test was used to analyze differences among competition treatment and location groups. For the intraspecific competition experiment, two-way ANOVA was used to analyze differences in flower and leaf number among species and locations. All analyses (and post-hoc analyses) were performed using SPSS (version 22, IBM Corp., Armonk, NY, USA).

3. Results

Competition treatment had inconsistent effects on mine site and adjacent nonmine plants across species (Figure 1). Location × competition effects were not significant in any species in terms of leaf number, flower number, or dry weight (Tables 1 and 2). *Anagallis* and *Senecio* had significant location effects—the mine site population contained a higher leaf number than that of the adjacent population across treatments (Table 3). Competition effect was significant in *Anagallis*, *Cirsium*, *Echium*, and *Oxalis*, where competition decreased leaf number (mine site: 43%, 9%, 25%, and 32% reduction, respectively; adjacent area: 55%, 16%, 16%, and 49% reduction, respectively). Plants with upright growth habits (i.e. *Conyza* and *Senecio*) were only modestly affected by the grass competitor, *P. monspeliensis*, where there were no significant competition effects on leaf number and dry weight (Table 1). The patterns observed in the dry weight data were consistent with the leaf and flower number observations (Table 3).

Location effect was significant for *Anagallis*, where mine site plants produced more than twice the number of flowers compared to adjacent plants (mine site average: 197.3 flowers; adjacent area average: 94.4; Table 3). Competition effect was significant for *Conyza* and *Oxalis*, where competition had negative effects on flower number (Table 2; Figure 2). Block effects were significant for leaf

number and dry weight, but not for flower number. SLA did not differ between mine and adjacent plants. The location × competition interaction effect was also not significant except in *Echium*. The competition treatment had a significant effect on SLA in *Conyza* and *Echium* species, where SLA tended to be higher under competition (Table 3). In *Echium*, SLA was significantly higher in mine site plants than adjacent area plants under low competition treatment (36% higher SLA) (Figure 3).

In the intraspecific competition experiment, there were overall significant differences among species. The species × location interaction effect was also significant; thus species were analyzed separately with one-way ANOVAs to detect differences in flower and leaf number. We found significant differences in flower number for *Anagallis* and *Conyza* populations, where the mine site plants expressed significantly greater performance than adjacent area plants. Conversely, *Oxalis* adjacent area plants had significantly greater performance than mine site plants (Table 4). In *Anagallis*, 2 out of 7 replicate adjacent area plants did not survive to the end of the experiment in the presence of competing mine site plants. Leaf number differences in *Oxalis* were also significant; adjacent area plants had higher numbers of flowers and leaves (Figure 4).

4. Discussion

We found that performance under competition was not lower in mine site plants compared to plants from the adjacent area, and the mine site plants did not have lower performance under intraspecific competition. As observed in the previous study, the species in this study did not demonstrate any trade-offs in performance traits when grown alone under low-stress conditions (Fazlioglu et al., 2017). A lack of differences in performance between mine site and adjacent area populations has been found before at other mine sites, in various studies (Antonovics, 1977; Harper et al., 1997; reviewed in Wan et al., 2017). However, these tended to focus on only one species inhabiting a particular site (e.g., Harper et al., 1997). Here we report that multiple mine site species did not express lower performance under competition. *Cirsium* and *Oxalis* mine plants had only slightly lower performance than adjacent plants under control treatment. The low magnitude of this decrease reflects the results of many previous studies (Harper et al., 1997; Dechamps et al., 2008; Wan et al., 2017).

Mine site plants of *Echium* expressed a relatively high SLA under low competition compared to adjacent area plants. This finding suggests mine site plants employed a faster growth strategy to maintain growth under competition where plants use fewer resources to produce thinner leaves with lower longevity. However,

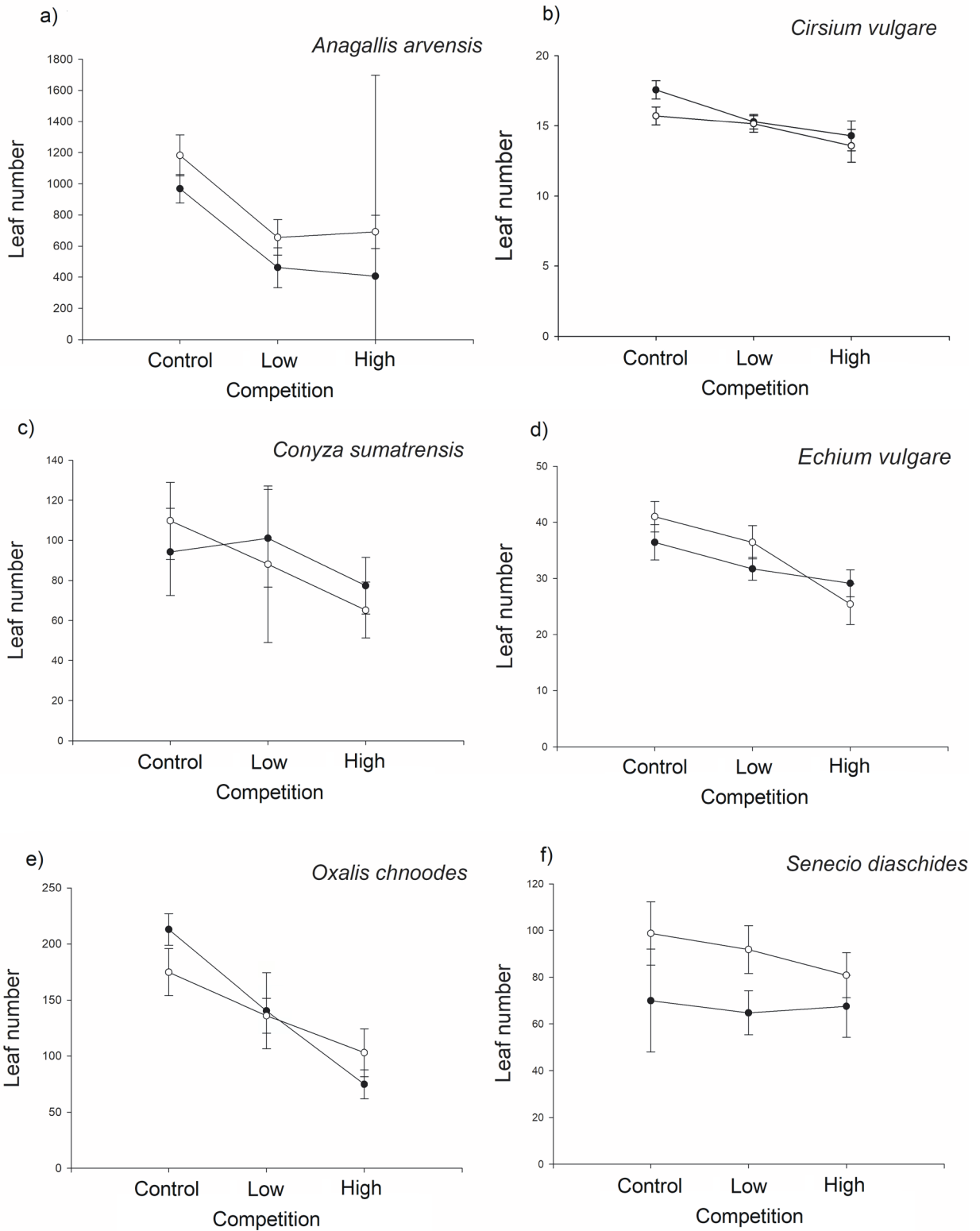


Figure 1. Mean leaf number (\pm SE) for each species (a–f) under control, low, and high competition treatments. Black and open circles represent populations from the adjacent area and from the mine site, respectively.

Table 1. The effect of location and competition treatment on performance traits across species.

Leaf number				
Source	df	SS	F	P
Species	5	5,610,220	168.20	< 0.0001
Competition	2	182,160	13.65	< 0.0001
Species × Competition	10	375,764	5.63	< 0.0001
Location[Species]	6	152,635	3.81	< 0.01
Location[Species] × Competition	12	25,995	0.32	0.98
Block	6	321,114	3.86	< 0.01
Flower number				
Source	df	SS	F	P
Species	3	3,820,294	91.91	< 0.0001
Competition	2	100,106	3.61	0.03
Species × Competition	6	359,360	4.32	< 0.001
Location[Species]	4	399,898	7.22	< 0.0001
Location[Species] × Competition	8	137,230	1.24	0.28
Block	6	13,854	0.35	0.91
Dry weight				
Source	df	SS	F	P
Species	5	54,488.90	103.64	< 0.0001
Competition	2	1365.17	6.49	< 0.01
Species × Competition	10	2342.38	2.23	0.02
Location[Species]	6	497.18	0.79	0.58
Location[Species] × Competition	12	57.41	0.05	1.00
Block	6	1635.14	2.59	0.02
SLA				
Source	df	SS	F	P
Species	5	204,575.78	148.83	< 0.0001
Competition	2	387.83	0.71	0.50
Species × Competition	10	8516.32	3.10	< 0.01
Location[Species]	6	1470.21	0.89	0.50
Location[Species] × Competition	2	544.62	0.99	0.37
Block	1	168.73	0.61	0.43

Note: df denotes numerator degrees of freedom. Bold P-values indicate significant differences at $P < 0.05$.

Table 2. The effect of location and competition on performance traits within species.

Trait	Source	<i>Anagallis</i>	<i>Cirsium</i>	<i>Conyza</i>	<i>Echium</i>	<i>Oxalis</i>	<i>Senecio</i>
		P	P	P	P	P	P
Leaf number	Location	0.03	0.18	0.87	0.43	0.79	0.04
	Competition	<0.0001	0.01	0.39	0.01	<0.001	0.74
	L × C	0.93	0.57	0.79	0.26	0.34	0.81
Flower number	Block	0.40	0.16	0.01	0.58	0.02	0.48
	Location	0.01	-	0.52	-	0.35	0.09
	Competition	0.08	-	0.02	-	0.01	0.42
Dry weight	L × C	0.72	-	0.56	-	0.30	0.54
	Block	0.27	-	0.20	-	0.06	0.18
	Location	<0.01	0.32	0.83	0.32	0.90	0.14
Competition	Competition	<0.0001	0.18	0.09	<0.0001	0.31	0.23
	L × C	0.65	0.49	0.72	0.70	0.58	0.98
	Block	0.66	0.65	0.30	0.39	0.91	0.08

Note: Bold P-values indicate significant differences at $P < 0.05$.

these differences in SLA among the locations disappeared under high competition. Range-edge populations of the introduced *Plantago lanceolata* specialized to stressful habitats demonstrated a similar response under strong competition, a response that was absent in the ancestral unspecialized plants (Wan et al., 2016).

Under intraspecific competition, mine site plants had equal performance compared to adjacent plants except for *Anagallis* and *Oxalis*. Two out of seven replicates of *Anagallis* adjacent area plants did not survive to the end of the experiment in the presence of competing mine plants, and mine site *Anagallis* plants also had significantly more flowers than the remaining adjacent plants. Only mine site *Oxalis* expressed significantly lower performance than adjacent plants under intraspecific competition with plants from the adjacent area (e.g., Cook et al., 1972). Our companion study (Fazlioglu et al., 2017) used the same populations for most of the species (all species used here except for *Cirsium* and *Senecio*, with an additional *Hypochaeris radicata*), and found that the adjacent area plants did not have lower performance than the mine site plants under high acidity treatment. These findings are contrary to the expectation that mine plants are restricted to mine sites (Antonovics and Bradshaw, 1970; McNaughton et al., 1974; Hayes et al., 2003). The overall lack of evidence of trade-offs and specialization at this particular site suggests that in some cases generalist strategies could allow the colonization of extremely stressful areas.

Interestingly, *Anagallis* and *Senecio* mine site populations had significantly greater performance than adjacent area populations and did not have lower performance under competition. Stress-tolerant genotypes with greater performance than adjacent nonstress-tolerant genotypes are relatively uncommon (Antonovics, 1977; Kiang, 1982; Shu et al., 2002; Délye et al., 2013). For instance, in a metaanalysis, we compared the performance of mine site and herbicide-tolerant plants with the respective adjacent nontolerant plants. The results showed that there were only fewer than 6 out of 38 cases (<14%) for mine sites and fewer than 12 out of 40 cases (<23%) for herbicide tolerance where the stress-tolerant plants outperformed the nonstress-tolerant plants (Wan et al., 2017). However, even these tend to remain restricted to their habitats. For instance, the Ile-1781-Leu ACCase herbicide-resistant populations are locally restricted, even though they consistently have higher performance than the wild-type genotypes and the other mutants (see Figure 3 of Delyé et al., 2013). The overall lack of trade-off in performance in mine site plants, including under inter- and intraspecific competition, suggests that other factors such as demographics (e.g., density dependence) or genetic restraints (e.g., a low fitness hybrid valley) may be limiting the spread of these mine site populations (Gomulkiewicz and Houle, 2009). In particular, the higher performing mine site *Anagallis* and *Senecio* populations may be restricted due to these unmeasured factors.

Table 3. Trait means for each species from each location and treatment (\pm SE).

Species	Location	Competition treatment	Leaf number	Flower number	Flowering time (days)	SLA (mm ² /mg)	Dry weight (g)
<i>Anagallis</i>	Mine	Control	1181.4 \pm 131.2	291.7 \pm 76.7	85.6 \pm 8.4	71.0 \pm 11.5	13.4 \pm 1.8
		Low	656.0 \pm 114.8	144.9 \pm 35.2	96.6 \pm 3.3	66.9 \pm 7.7	6.3 \pm 1.1
		High	691.4 \pm 106.2	155.4 \pm 58.7	100.3 \pm 2.3	82.2 \pm 9.9	4.8 \pm 1.5
	Adjacent	Control	967.7 \pm 90.7	138.1 \pm 50.7	88.7 \pm 7.8	71.7 \pm 12.1	8.4 \pm 1.9
		Low	461.4 \pm 129.4	66.7 \pm 58.2	98.0 \pm 2.4	80.8 \pm 19.0	2.9 \pm 0.9
		High	406.4 \pm 1290.0	78.3 \pm 38.9	95.7 \pm 1.54	107.1 \pm 12.9	2.5 \pm 0.9
<i>Cirsium</i>	Mine	Control	15.7 \pm 0.6	-	-	9.5 \pm 0.6	9.5 \pm 1.3
		Low	15.1 \pm 0.6	-	-	10.0 \pm 0.8	7.6 \pm 0.9
		High	13.6 \pm 1.2	-	-	9.7 \pm 0.4	6.2 \pm 0.9
	Adjacent	Control	17.6 \pm 0.7	-	-	9.0 \pm 0.4	8.8 \pm 1.1
		Low	15.3 \pm 0.5	-	-	9.1 \pm 0.4	9.6 \pm 1.4
		High	14.3 \pm 1.1	-	-	9.8 \pm 0.4	7.8 \pm 1.0
<i>Conyza</i>	Mine	Control	109.7 \pm 19.3	219.9 \pm 30.2	96.6 \pm 3.9	12.8 \pm 0.9	50.2 \pm 9.5
		Low	88.0 \pm 39.1	185.9 \pm 37.2	101.1 \pm 2.9	14.0 \pm 1.3	52.7 \pm 13.3
		High	65.1 \pm 14.0	57.1 \pm 23.4	97.3 \pm 6.7	17.5 \pm 1.9	35.3 \pm 7.9
	Adjacent	Control	94.2 \pm 21.7	206.3 \pm 53.2	98.7 \pm 3.6	13.0 \pm 1.7	47.9 \pm 4.0
		Low	101.0 \pm 24.4	192.3 \pm 58.9	97.0 \pm 3.5	14.9 \pm 1.7	50.2 \pm 9.0
		High	77.3 \pm 14.1	131.0 \pm 40.8	102.9 \pm 2.4	17.4 \pm 1.4	30.1 \pm 5.5
<i>Echium</i>	Mine	Control	41.0 \pm 2.7	-	-	9.9 \pm 0.5	5.2 \pm 0.7
		Low	36.4 \pm 3.0	-	-	12.4 \pm 0.8	3.8 \pm 0.6
		High	25.4 \pm 3.6	-	-	11.7 \pm 0.9	2.1 \pm 0.6
	Adjacent	Control	36.4 \pm 3.2	-	-	10.0 \pm 0.7	5.5 \pm 0.4
		Low	31.7 \pm 2.0	-	-	9.2 \pm 0.5	3.8 \pm 0.4
		High	29.1 \pm 2.4	-	-	12.4 \pm 0.8	3.0 \pm 0.7
<i>Oxalis</i>	Mine	Control	174.8 \pm 20.9	52.7 \pm 7.9	30.4 \pm 2.5	91.5 \pm 24.7	3.3 \pm 0.5
		Low	136.0 \pm 15.7	26.6 \pm 6.7	29.0 \pm 2.8	65.1 \pm 3.0	2.8 \pm 0.8
		High	103.1 \pm 21.2	36.4 \pm 13.7	40.7 \pm 11.2	57.4 \pm 4.4	2.7 \pm 0.8
	Adjacent	Control	212.8 \pm 14.0	50.3 \pm 7.6	30.3 \pm 2.7	79.0 \pm 7.4	4.4 \pm 0.6
		Low	140.4 \pm 34.0	30.4 \pm 7.6	25.4 \pm 2.6	70.7 \pm 3.8	3.3 \pm 1.3
		High	74.9 \pm 12.9	14.6 \pm 5.0	28.8 \pm 2.6	54.7 \pm 6.5	1.9 \pm 0.7
<i>Senecio</i>	Mine	Control	98.7 \pm 13.6	491.6 \pm 77.9	95.4 \pm 1.4	14.8 \pm 1.0	26.1 \pm 4.5
		Low	91.8 \pm 10.2	502.5 \pm 80.7	92.3 \pm 1.5	14.8 \pm 1.2	33.2 \pm 3.9
		High	80.9 \pm 9.6	514.6 \pm 83.0	96.9 \pm 4.9	15.6 \pm 1.4	24.9 \pm 3.1
	Adjacent	Control	70.1 \pm 22.0	336.7 \pm 47.9	94.0 \pm 1.2	11.8 \pm 0.5	21.2 \pm 5.8
		Low	64.8 \pm 9.5	295.0 \pm 49.9	87.2 \pm 4.7	14.7 \pm 1.6	27.3 \pm 1.9
		High	67.6 \pm 13.3	495.5 \pm 139.0	98.8 \pm 2.5	16.0 \pm 2.7	21.2 \pm 7.1

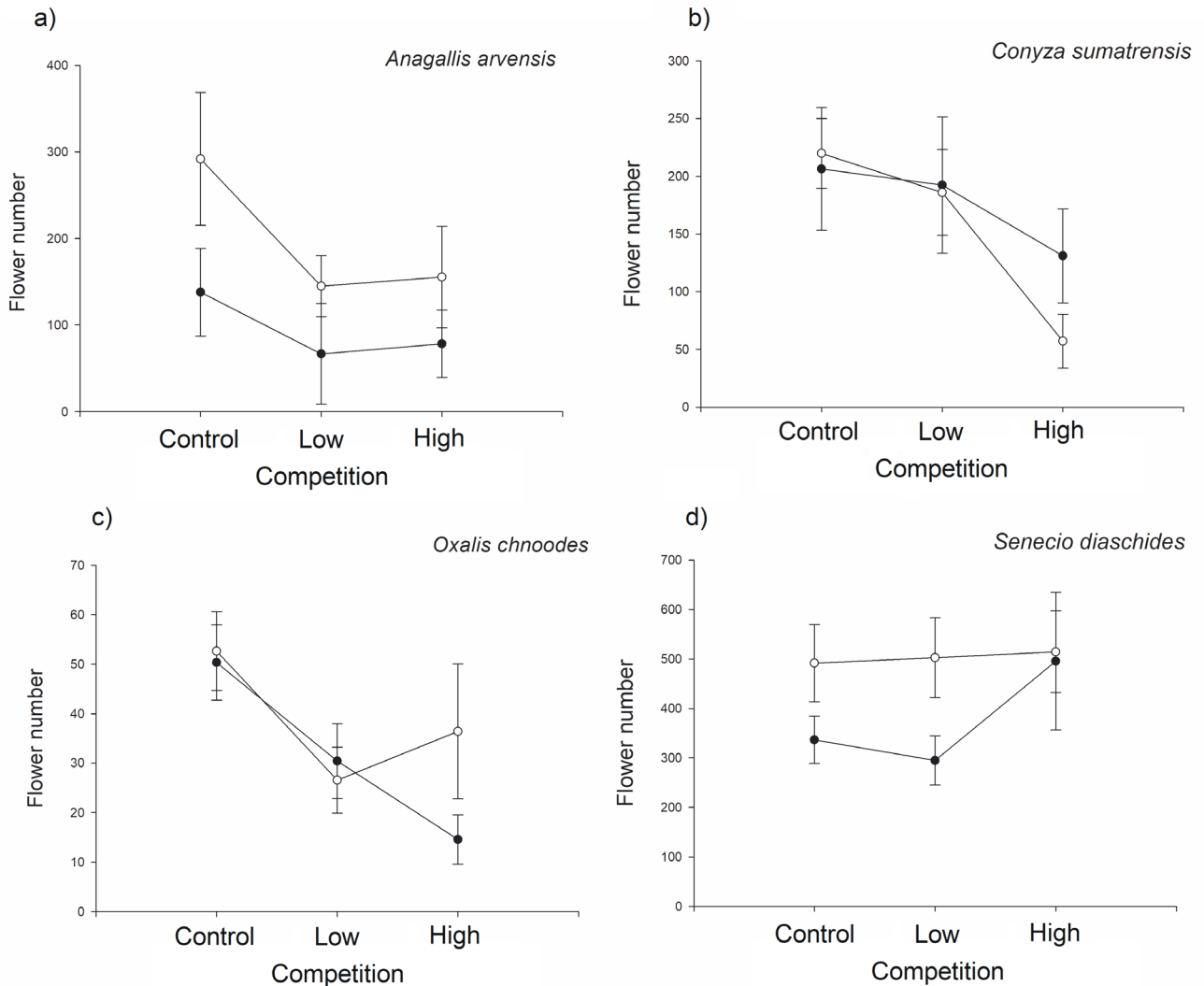


Figure 2. Mean flower number (\pm SE) for each species reaching reproduction (a–d) under control, low, and high competition treatments. Black and open circles represent populations from the adjacent area and mine site, respectively.

Hayes et al. (2003) assessed the metal tolerance of *Verbascum virgatum* (not used in the present study) and *Acacia dealbata* (not found inside the mine site) inhabiting the run-off stream extending from the mine site. Similar to the findings of our two experiments, they found the populations from nearby adjacent area soils also had considerable metal accumulation abilities, suggesting that these populations are extreme generalists readily capable of colonizing the mine site. High plasticity in physiology and functional traits may have facilitated the colonization and may mask specialization (Baquedano et al., 2008). These results are contrary to studies documenting the sharp clinal separation of mine site habitat boundaries

among mine site and adjacent area genotypes, as is often the case in studies detailing the specialization of mine site plants (Antonovics and Bradshaw, 1970; Posthuma and Van Straalen, 1993; Antonovics, 2006). In addition, these results are contrary to the expectation that mine site specialists have poorer performance under interspecific and/or intraspecific competition, compared to generalists (Warwick and Black, 1981; Gassman and Futuyma, 2005). In some cases, the stress genotype may outcompete the nonstress genotype (Antonovics, 1977). These findings (as well as Fazlioglu et al., 2017) provide support to the idea that colonization of extremely stressful environments may not necessarily involve specialization (Che-Castaldo

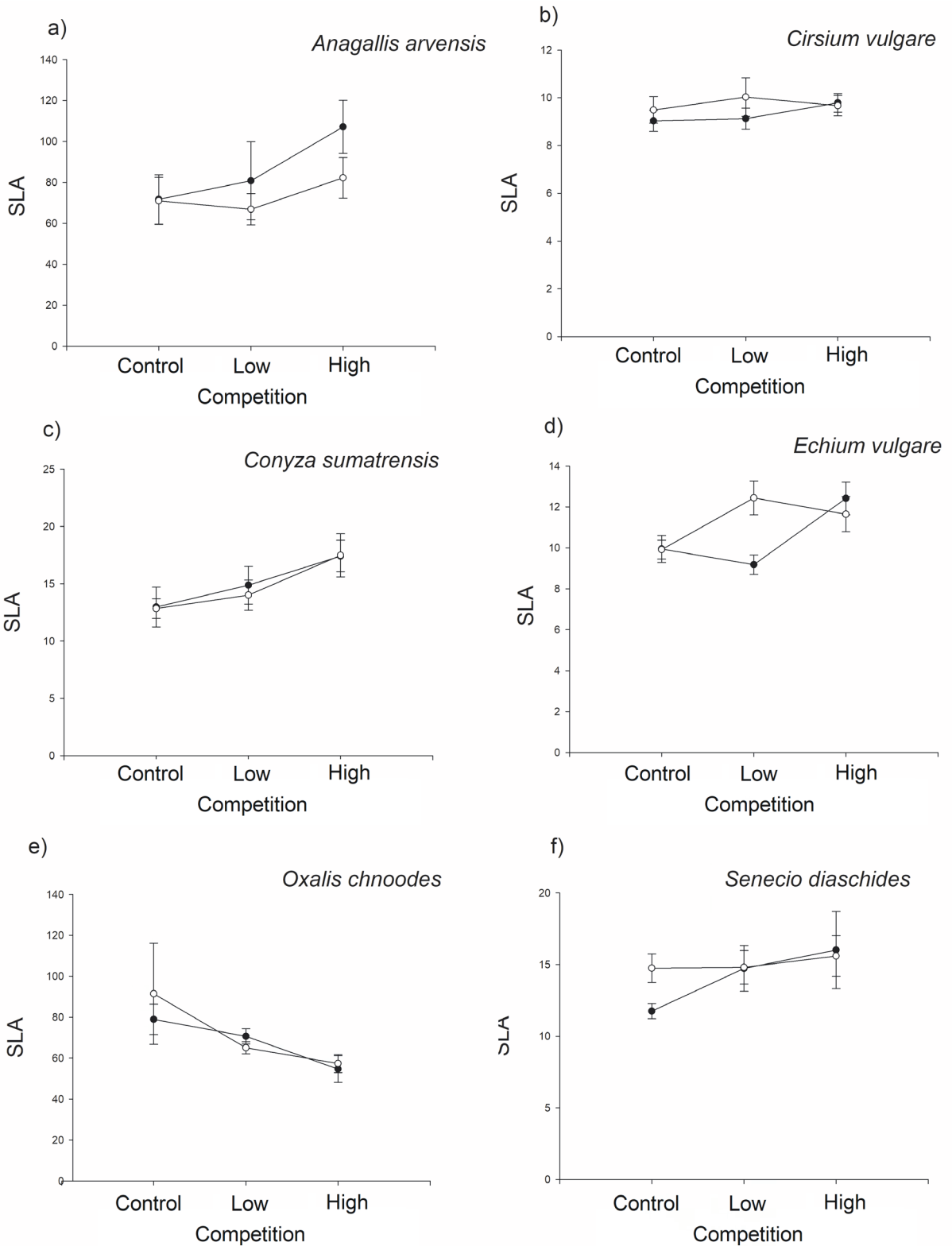


Figure 3. Mean SLA (±SE) for each species (a–f) under control, low, and high competition treatments. Black and open circles represent populations from the adjacent area and mine site, respectively. Within treatments, differences that were significant among locations are marked with an asterisk.

Table 4. The effect of location (mine and adjacent) on performance traits for the intraspecific competition trial.

<i>Anagallis arvensis</i>				
	df	SS	F	P
Flower number	1 (n = 5)	238,163	6.149	0.03
Leaf number	1 (n = 5)	37,210	1.186	0.31
<i>Conzuya sumatrensis</i>				
	df	SS	F	P
Flower number	1	24,528	9.98	0.01
Leaf number	1	193.14	1.39	0.26
<i>Echium vulgare</i>				
	df	SS	F	P
Leaf number	1	10.29	0.48	0.50
<i>Oxalis chnoodes</i>				
	df	SS	F	P
Flower number	1	540.64	7.36	0.02
Leaf number	1	8501.79	5.09	0.04
<i>Senecio diaschides</i>				
	df	SS	F	P
Flower number	1	23,563	4.15	0.11
Leaf number	1	60.17	4.57	0.10

Note: Bold P-values indicate significant differences at $P < 0.05$ (n = 7 unless otherwise indicated, df denotes numerator degrees of freedom).

and Inouye, 2015); plant responses to other environmental heterogeneity may be responsible for some of the phenotypic differences observed in previous studies.

Extreme generalists found in high metal habitats may be represented by pseudometallophytes (Antonovics, 1977; Remold, 2012). The species at this mine site represent a fraction of the species pool in the surrounding area. Therefore, it is possible that the mine site species already had tolerances allowing them to invade the site (i.e. standing genetic variation). In an experimental study, Antonovics (2006) found that reproductive isolation brought on by different flowering times can drive genotypic isolation and specialization. In that study, plants expressed these differences under controlled conditions, and these differences had persisted through approximately 40 years after colonization, suggesting that ecotypic divergence had occurred (Antonovics, 2006). In our case, the mine site and adjacent area plants started flowering at the same time (Table 3). High gene flow among populations could

result in the spread of the specialized mine site genotypes (Busi et al., 2011). It could not be established at this stage whether the low-stress populations are extreme generalists or the surrounding populations retain some individuals that are already preadapted to the stressors before meeting the mine site habitat. Che-Castaldo and Inouye (2015) assessing trade-offs in hyperaccumulator species found that abiotic factors may have higher importance over competitive effects in limiting their spread. Therefore, hyperaccumulator species may establish outside mine sites given that abiotic factors are not limiting.

In conclusion, plants inhabiting a mine site may have lower performance under competition associated with specialization. Contrary to this prediction, our results suggest that, under some circumstances, multiple species may colonize high-stress habitats from low-stress habitats without the predicted consequences of specialization, such as reduced competitive ability. The insights gained from these works have potentially important implications in

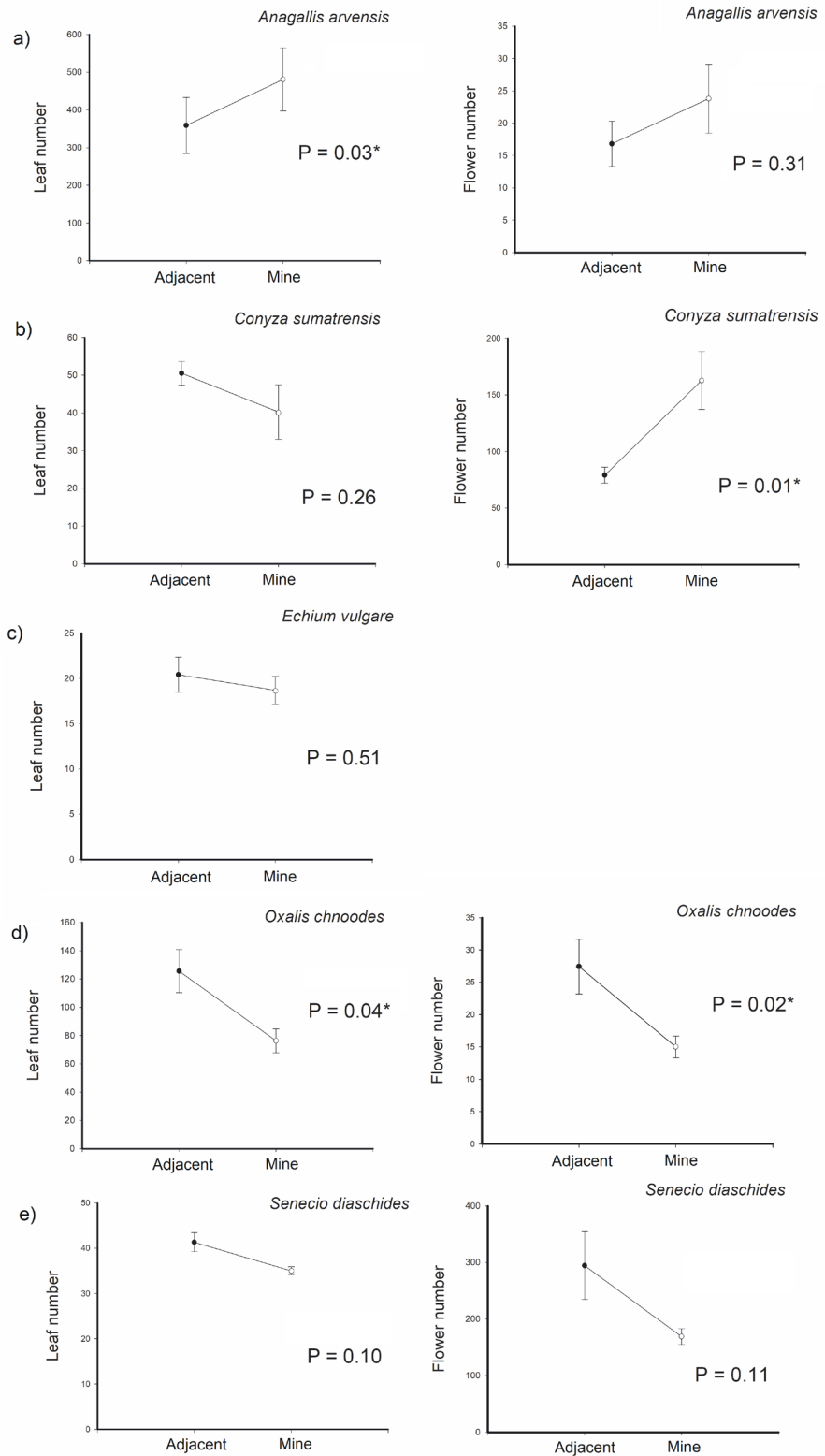


Figure 4. Mean leaf number and flower number (\pm SE) for intraspecific competition treatments. Significant differences among mine site and adjacent area pairs are marked with an asterisk.

predicting colonization ability under novel stresses. In particular, future studies can uncover the conditions in which plant species can colonize an extremely stressful environment without specialization and reduced performance under competition.

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