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Marjolein Visser^a, Perrine Collin^a, Azaiez Ouled Belgacem^b & Mohamed Neffati^b

^a Ecole Interfacultaire des Bioingénieurs, Université Libre de Bruxelles, Brussels, Belgium

^b Range Ecology Laboratory, Institute of Arid Regions, Médenine, Tunisia

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***Argyrobium uniflorum* Seedlings Respond Strongly to Small Doses of Phosphorus: Consequences for Rehabilitating Degraded Arid Fallows in Presaharian Tunisia**

Marjolein Visser¹, Perrine Collin¹, Azaiez Ouled Belgacem², and Mohamed Neffati²

¹Ecole Interfacultaire des Bioingénieurs, Université Libre de Bruxelles, Brussels, Belgium

²Range Ecology Laboratory, Institute of Arid Regions, Médenine, Tunisia

*In North African dryland research, few recognize that phosphorus (P) is the main soil nutrient limiting plant growth for legumes, in particular. P-fertilisation of legume reseedings could boost biological N-fixation and N-yield through enhanced biomass production, which, in turn, can benefit the re-establishment of resource-responsive perennial grasses. This is the hypothesis underlying a short-term fertilization experiment carried out with seedlings of *Argyrobium uniflorum*, a native palatable legume. A secondary question was whether plant-available P, as measured by the Olsen method, is the best way to assess P-status in arid, calcareous, and nutrient-depleted soils that are renowned for their resource patchiness. Results show a significant effect of phosphorus addition on dry matter and N-yield. Seedlings responded to 25-kg P ha⁻¹ by almost doubling dry matter production, yet no further improvement was observed for 50-kg P ha⁻¹. The better plant growth corresponded with higher numbers of active nodules on P-fertilized seedlings. Plant P-content showed a positive and significant linear correlation with dry matter yield, whereas soil total and Olsen P-content did not, suggesting that plant P-content might be a more sensitive indicator of plant-available P than Olsen soil P-content. The observation that nodulation was efficient even though the land had not carried any legumes for over twenty years suggests that reseedling is feasible without inoculation. The strong response of *Argyrobium uniflorum* seedlings to modest doses of phosphorus confirms the role of both legumes and phosphorus as key factors for restoring degraded drylands.*

Keywords degradation, dryland cereal fallows, native legumes, rehabilitation, soil fertility

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Address correspondence to Marjolein Visser, Ecole Interfacultaire des Bioingénieurs, Université Libre de Bruxelles, CP 169, 50, Avenue Franklin Roosevelt, 1050 Brussels, Belgium. E-mail: marjolein.visser@ulb.ac.be

Aridity is often associated with low soil fertility in North Africa. However, prior to human impact, these soils were favorable to plant growth. Despite the Mediterranean dry climate, open forest was then the prevailing plant formation. Soil fertility decline was the long-term result of loss of plant cover, which is a manmade process. Today these same soils are characterized by particularly low organic matter, plant-available nitrogen, phosphorus content, and Cation Exchange Capacity (CEC). This lack of soil fertility is an often overlooked bottleneck in land restoration, rehabilitation, or reclamation programs in North Africa and beyond (Roose et al., 2011). Soil phosphorus (P) is the least mobile macronutrient, and in calcareous drylands in particular, it is prone to quick and irreversible fixation as various Ca-phosphates, either following application as a fertilizer or upon decomposition of biomass. The total P-pool is thus several times larger than the plant-available P-pool, and, moreover, the disappearance of perennial plant root activity from the plant-root system leads to more fixed and less plant-available P in the total P-pool. This is why P has now become limiting in most calcareous soils of North Africa (Ryan, 1983; Matar et al., 1992). Studies show that, next to aridity, P-deficiency is the second-most important reason why the symbiosis between various legumes and *Rhizobium* sp. is underperforming. The response to P-addition can be spectacular. Research in situations similar to those in Tunisia has shown that P significantly boosts rangeland and legume productivity (Osman et al., 1991; Ewing, 1999).

The neglect of the arid soil fertility problem means that little is known of the responsiveness of North-African steppe legumes to P-addition. This is the case in particular for *Argyrobium uniflorum* (Decne.) Jaub. & Spach, a pluriannual herbaceous legume of high pastoral value. As a widespread interstitial species of the *Rhanterium suaveolens* steppes in sandy plains in North Africa, it is considered the most promising complement to highly palatable native steppe grasses such as *Stipa lagascae* R. & Sch. and *Cenchrus ciliaris* L. that need a source of nitrogen to express their productive potential (Jauffret & Visser, 2003). Yet, little is known about the potential of this species and its ability to nodulate in degraded sites where legumes have been absent for many years. A practical problem is to find nodules on established individuals because in mature plants nodules occur on deep roots and are easily separated from the roots when dug up.

In North Africa, dryland restoration through reseedling has experienced high failure rates for a host of reasons (Visser et al., 2011). Technically, to avoid failure with perennials, it is essential to foster substantial seedling growth during the establishment year because this directly determines seedling survival beyond the first summer drought (Visser et al., 2010). This means that any rain event must release enough N and P for uptake by seedlings with limited root systems. Beyond the seedling stage and when reseedling legumes and grasses, adding small but well-timed and placed amounts of external P might prove a key to the survival of both.

As a first step toward exploiting the potential of *Argyrobium uniflorum* in dryland restoration, this short-term experiment studies seedling response to P-addition through parameters relating to nutrient content, biomass, and nodulation in near-rainfed conditions in arid Tunisia. The prime objective was to check what had been found elsewhere in the same eco-region with the Olsen method to measure plant-available soil P (Osman et al., 1991; Matar et al., 1992; Ewing, 1999): (1) a critical lower level of 10 ppm plant-available soil P, below which the vegetation is expected to respond significantly to P-addition; and (2) a saturation level of about 20 ppm plant-available soil P, above which the vegetation ceases to respond to further P-addition.

Materials and Methods

The P-fertilization trial was carried out in the experimental garden of the Institute of Arid Regions in Médenine (33°30' N and 10°40' E), 22 km east of Médenine and 5 km inland from the coast, at low elevation (about 25 m above sea level). The climate is presaharian: very arid but not desertic (average annual rainfall between 100 and 200 mm) with mild winters (lowest monthly minimum is between 5 and 7°C) and an average annual temperature of 20°C (Floret & Pontanier, 1982). A lime-rich sandy-loamy upper horizon overlays a gypsous crust at an average depth of 40 cm. Conventional soil fertility parameters all present extremely low values on this type of steppic soils. Typically, organic matter content is <0.6%, total nitrogen content <0.05%, CEC between 5 and 8 meq 100 g⁻¹ and plant-available soil P-content (or Olsen P, in short, as determined by the method of Olsen) is <10 ppm, whereas both lime (10–25%) and gypsum (1–8%) are always present even outside crusts (Floret & Pontanier, 1982). For the trial, land was chosen that had remained fallow for over 30 years after 20 years of barley cropping without the addition of any fertilizer, organic or inorganic. Weed buildup was reduced by annual disk-plowing.

The trial ran from October 2007 to March 2008. Total rainfall during that period was 140 mm (close to the theoretical annual average of the upper and lower isohyets defining presaharian Tunisia), but most of that rain fell in January. Three levels of P-addition (a control of 0 kg P ha⁻¹, an intermediate level of 25 kg P ha⁻¹, and an upper level of 50 kg P ha⁻¹) were applied in a randomized complete block design with four replications. The levels were chosen on the basis of a trial on Syrian grassland dominated by annual legumes (Osman et al., 1991). The trial was laid out in an area of 300 m², subdivided in twelve main irrigation basins, 2 m wide x 4 m long each, at spacings of 1 m. The P was applied as triple superphosphate (26.47% P), the most available P-carrier on the Tunisian market. Prior to the treatments, the basins were flooded with low-salinity water. Triple superphosphate was then broadcast by hand.

Some precautions were necessary to foster good emergence and uniform stands. The seed was harvested 100 km north-west of Médenine (similar ecological conditions) in May 2007 and stocked in the seed bank of the IRA (laboratory conditions). Prior to seeding, the seed was scarified by soaking in H₂SO₄ (diluted at 70%) for 90 minutes. This treatment was found to yield the best germination capacity (88%) on this particular seed lot (Collin, 2008). Seed was hand sown into furrows of 1-cm depth on 15 October 2007, three days after P-addition. There were eight furrows per basin, 2-m long and spaced 50 cm. Seeding density was high and stood at approximately 850 seeds per furrow (425 seeds m⁻¹). The plots were then hand watered with a hose to avoid seed drifting. This type of irrigation was repeated at day 10, 25, 30, 35, and 45 after seeding. On 10 December 2007, the rows were thinned at 20 seedlings spaced 10 cm apart. After that date, the seedlings were rainfed with no supplementary irrigation. No weeding was done. A composite soil sample at 15-cm depth of each of the 12 plots was obtained, dried at 105°C and sieved. Total soil P-content was determined by digestion with HClO₄ (Pauwels et al., 1992) and plant-available P-content was determined with the Olsen method (Van Ranst et al., 1999).

The seedlings were harvested on 26 March 2008. The ten central seedlings of two contiguous furrows were fully uprooted. With spades, two trenches 30-cm deep were dug at each side of the furrows. Each individual plant was carefully dug up with a

gardening spade. The soil cylinder attached to the roots was carefully removed in a basin with water. Several observations on the root system were made as plants were dug up (FAO, 1985): (1) Total number of nodules; (2) Nodule color (white, red, or brown); (3) nodule longest and shortest diameter (with a 1-mm precision ruler) ; (4) nodule position on the root: main pivot (considered more efficient) or secondary roots (considered less efficient); and (5) nodule depth as measured from the plant collar along the pivot with a 1-mm precision rule.

The aboveground and underground parts of each individual plant were weighed in the laboratory (0.01 g precision balance), oven-dried (60°C for 48 h), and weighed again to determine dry matter yield. Individual plants were pooled per plot to determine nutrient content. Plant P-content was determined by the method of Scheel (Pauwels et al., 1992; Van Ranst et al., 1999). Plant N-content was determined with a C/N automatic analyzer. With the resulting total dry matter (DM) harvest per plot and N-content, N-yield could be calculated as total DM x N-content.

Analysis of variance (with experimental blocks added as an extra source of variation) was applied on six individual variables: DM-yield, total soil P-content, plant-available P-content, plant P-content, plant N-content, and plant N : P ratio. The correlation patterns between these variables and one extra variable (number of colored nodules considered active) were analyzed with Principal Components Analysis (PCA). All statistical analysis has been carried out with SPSS 17.0.

Results

Assuming the perfect mixing of the added P in the upper 15 cm of the soil (sampling depth) and a soil bulk density of 1.5 kg dm⁻³, a dose of 25-kg P ha⁻¹ is predicted to add 11.1 ppm. The comparison of the effects of the three P-levels with a simple set of soil and plant variables shows that these modest P-doses had big effects (Table 1).

This is observed first for plant-available soil P-content. The control plots show plant-available soil P-levels similar to those obtained elsewhere in the region (Ameloot, 2001). On average, the control plots have 8.2 ppm plant-available P. These levels increase significantly with P-addition, up to 21.8 ppm in the 50-kg P ha⁻¹ treatment. The response of plant-available soil P is in stark but logical contrast with the lack of significant response of total soil P. Adding roughly 11 ppm of P by

Table 1. Main results of analyses of variance of six plant and soil parameters in response to phosphorus fertilization of *Argyrolobium uniflorum*

Variables	VC (%)	Phosphorus dose (kg ha ⁻¹)			P-value
		0	25	50	
Total soil P (ppm)	16.0	146.8	151.2	178.2	0.136
Plant-available soil P (ppm)	16.3	8.2	13.8	21.8	0.001
Plant P (ppm)	6.6	856	1072	1037	0.002
Plant N (ppm)	11.9	20054	20053	19588	0.941
N:P in plant (unitless)	11.6	23.5	18.7	19.0	0.074
Dry Matter (kg ha ⁻¹)	16.0	20.0	37.9	33.3	0.006

Note. VC: variation coefficient.

broadcasting granulated P-fertilizer to a variable 150 ppm already present (variation coefficient of 16%), was expected not to significantly increase total soil P.

More importantly, big effects are observed at the plant level. The significant increase in plant-available P is indeed reflected in the increase of plant P-content (variation coefficient only 6%) as well as in the increase of plant DM. But the dose-response curve of both parameters levels off after the first 25-kg P ha⁻¹ (Table 1). Because the added P has not changed the N-content of the plants, the increase in N-yield is the direct result of the increase in DM-yield.

Few nodules were observed on the seedlings; on average, not even one per seedling. Perhaps as a consequence of these low numbers, mean nodule numbers per type (colored or white) did not show significant differences between treatments when compared with a Kruskal-Wallis test. The observations relating to nodule quality did not show any differences between treatments either. However, when nodule numbers were totalled per treatment and these totals converted to proportionate representation between treatments, comparatively few colored nodules (19% of the total) were observed in the control plots, versus 40% in the 25-kg P ha⁻¹ plots and 41% in the 50-kg P ha⁻¹ plots. All nodules were observed on secondary roots at an average depth of 79 ± 27 mm. White nodules had regular shapes most of the time whereas colored nodules were mostly irregularly shaped. On average, they were 3 ± 2 mm long and 2 ± 1 mm wide.

The PCA on the seven variables (Figure 1) concentrated 43% of the total variation on the first axis (PC1), 20% on the second axis (PC2), and another 17% on the third axis (all with eigenvalues >1). The loading plot of PC2 on PC1 summarizes 63% of the total variation of the dataset (Figure 1a). A majority of variables has significant loadings on PC1. Plant available P-content, plant P-content, plant DM, and number of colored nodules (positive side of PC1) are thus well and positively correlated. Together they correlate negatively with N:P ratio, even if plant N-content is

Figure 1. (a) Loading plot of variables used in PCA and (b) score plot of individual values on the first two Principal Components (PC1 and PC2). PC1 and PC2 explain 43% and 20%, respectively, of the total variation. (a) Ptot: total soil P-content; Plsen: plant-available soil P-content; Pplant: plant P-content; Nplant: plant N-content; NPplant: N:P ratio in plant; DM: Dry Matter of *Argyrolobium uniflorum* seedlings; Coloured: number of colored nodules totalled over ten seedlings per plot. (b) White dots 0 kg P ha⁻¹; grey dots 25 kg P ha⁻¹; black dots 50 kg P ha⁻¹.

shown as varying independently from this group of correlated variables. This means the plant P-content rather than the plant N-content reacts to P-addition and lowers the N:P ratio. When the 12 scores of the treatment replicates are projected on the (PC1, PC2) plane (Figure 1b), the control plots are neatly clustered at the negative side of PC1 whereas the plots having received P are more widely spaced yet all but one situated at the positive side of PC1. The 25-kg ha⁻¹ doses are intermingled with the 50-kg ha⁻¹, thus confirming in a multivariate way the one-way analyses of variance: the response to P-addition levels off >25-kg P ha⁻¹.

However, a different result is observed at the bivariate relationships between DM-yield and the three different parameters measuring P-content of the soil-plant system without aggregating data over treatments (Figure 2). Depending on the type of P-content measured, there was either no linear relationship at all with DM-yield (Olsen P and total P), or there was a linear, as opposed to an asymptotic, relationship (plant P-content). Bivariate linear correlation analysis (Pearson) between those four dependent variables reveals that only the correlation coefficient between plant P-content and DM-yield is significantly positive (exact values reported under Figure 2). Figure 2 illustrates three important points: (1) Under the particular conditions of this experiment, and at this particular stage of plant development, plant P-content has not yet reached a ceiling at 50 kg P ha⁻¹; (2) In these extremely P-depleted arid soils, there is a problem with the precision and accuracy of the measurement of plant-available soil P; and (3) The coefficients of variation of the soil parameters are higher than those of the plant parameters (also shown in Table 1).

Figure 2. Dry matter produced by *Argyrolobium uniflorum* seedlings at the end of the experiment as a function of three measures of phosphorus in the soil-plant system: Olsen (crosses), total soil (circles), and total plant (diamonds). Corresponding Pearson correlation coefficients are: DM-yield & Olsen P 0.399 ($r=0.198$), DM-yield and total soil P 0.124 ($r=0.700$), DM-yield and total plant P 0.786 ($r=0.002$). Labels show P-dosis (0-25-50 kg P ha⁻¹).

Thus the small-scale patchiness of total and plant-available P influences plant P-content too, irrespective of the particular P-addition level. Therefore plant P-content might be a better reflection of plant-availability of P in the immediate vicinity of the individual seedlings than total or plant-available P as measured in composite soil samples.

Discussion

We undertook a small-scale and short-term experiment with seedlings of a plurianual legume native to the North African steppe to check its response to P-addition and the relevance of Olsen P as a measure of plant-availability of P in P-depleted dryland soils. We wanted to check in particular the lower and upper limits of 10 and 20 ppm of plant-available soil P of previously established dose-response curves. The results confirm these values in this particular case of row-seeded plantlets with a limited root system and high nutrient demand in response to hand-broadcast triple superphosphate. The strong response of these young plantlets can only mean plant-available soil P is a severely limiting plant growth factor. Beyond this result, the usefulness of plant-available P as determined by the Olsen method in this context might be questioned, since the levels of both total P and its plant-available fraction (Olsen P) are both low and spatially highly variable. There are two interrelated problems: (1) the conventional way of soil sampling and (2) the detection limit. (1) We proceeded with composite soil sampling because this is what soil laboratories prescribe; yet, in conditions of high small-scale patchiness of soil parameters in nutrient-depleted drylands, conventional soil sampling overlooks what is happening near the plant roots. However, analyzing a high number of individual soil samples is too cumbersome and costly to be a realistic alternative to composite sampling. (2) Olsen P-values measured on samples of non-enriched calcareous soils commonly approach the detection limit of 1 ppm. Near the detection limit an absolute deviation of 1 ppm on a reading of 10 ppm means a relative jump of 10%, whereas that same deviation of 1 ppm on a reading of 100 ppm means of relative jump of only 1%. Therefore, the accuracy (is it exact?) and precision (is it repeatable?) of the measurement suffer when working near the detection limit. Lack of accuracy and precision when measuring Olsen-P in P-poor soils means it may not be the best parameter to assess P-availability in this context.

In view of our results with plant P, which point to continuing P-limitation even at the highest dose of added P, it would be highly useful to check whether plant P is a better indicator of P-limitation than Olsen P. Beyond working with just the absolute values of plant P, the N:P ratio of plant DM has already been suggested as a good tool to measure N versus P-limitation in wetland vegetation (Verhoeven et al., 1996; Güsewell & Koerselman, 2002). Apart from improved precision, which provides less frustration about non-significance, one important advantage of plant-P as opposed to soil-P is that it is straightforward. There is only one P-content of a particular plant sample as opposed to many possible plant-available P-contents of the soil. In addition, plant nutrient extraction is easier than soil nutrient extraction. Samples are less heavy, easier to dry and to mix, and easier to transport. Arguments against working with plant-P are the prerequisite of live plant biomass at the moment of sampling and the possibility of interspecific differences in critical plant-P depending on, for example, life history strategies. However, these should be considered as a matter worth further study rather than an obstacle.

Conclusion

Argyrobium uniflorum is one of the few herbaceous pluriannual legumes of North Africa that are both persistent and highly palatable. Despite its potential to fulfill a key role in dryland rehabilitation programs, the species is under-researched, as much as is the role of phosphorus deficiency in this context. With this small-scale and short-term experiment, we demonstrate that doses of 25-kg ha⁻¹ or less have big effects on seedlings and that plant available soil-P as determined by the Olsen method might not be the most relevant indicator of P-limitation. Results suggest that the intelligent remediation of this P-deficiency through the use of native, responsive, and complementary grass-legume seed mixtures could help overcome the obstacles to restore dryland functioning.

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