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Phosphorus use efficiency of *Trifolium subterraneum* as affected by seeding rate under rainfed conditions

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Abstract. Legumes are particularly important feed sources for livestock due to their high nutritive value. The objective of this study was to examine the agronomic phosphorus use efficiency (PUE) of *Trifolium subterraneum* at different seeding rates (13, 17, 20 kg ha^{-1}) and phenological stages (inflorescence, fruiting). The experiment was conducted in Orestiada, northern Greece under rainfed conditions in 2014. Seeds were sown in February and phosphorus fertilization was applied before the seeding. According to the results agronomic PUE was higher at the inflorescence stage compared to the fruiting one. The lowest value of agronomic PUE was observed at the 17 kg ha^{-1} seeding rate, while there was no significant difference between the other seeding rates. However, all the corresponding values were negative. Positive agronomic PUE values were recorded at the seeding rate of 13 kg ha^{-1} during the inflorescence stage and at 20 kg ha^{-1} during the fruiting stage, indicating that phosphorus fertilization is essential to improve forage yield at the specific seeding rates.

Keywords. Forage production – Subterranean clover – Inflorescence – Fruiting – Fertilization.

Efficacité de l'utilisation du phosphore du trèfle souterrain (*Trifolium subterraneum*) influencé par le taux de semis en conditions pluviales

***Résumé**. Les légumineuses sont des sources d'alimentation particulièrement importantes pour le bétail en raison de leur haute valeur nutritive. L'objectif de cette étude était d'examiner l'efficacité agronomique de l'utilisation du phosphore (PUE) du trèfle souterrain à différents taux de semis (20, 17, 13 kg ha^{-1}) et stades phénologiques (inflorescence, fructification). L'expérience a été menée à Orestiada, au nord de la Grèce, dans des conditions pluviales au cours de 2014. Les graines ont été semées en février et la fertilisation phosphatée a été appliquée avant l'ensemencement. Selon les résultats la PUE agronomique était plus élevée au stade de l'inflorescence par rapport à la fructification. La valeur la plus basse de PUE agronomique a été observée au taux de semis 17 kg ha^{-1}, alors qu'il n'y avait pas de différence significative entre les autres taux de semis. Cependant, toutes les valeurs correspondantes ont été négatives. Des valeurs de PUE agronomiques positives ont été enregistrées à 13 kg ha^{-1} lors de l'étape de l'inflorescence et à 20 kg ha^{-1} pendant la phase de fructification, ce qui indique que la fertilisation phosphatée est essentielle pour améliorer le rendement en fourrage dans les taux de semis spécifiques.*

***Mots-clés.** Production de fourrage – Trèfle souterrain – Inflorescence – Fructification – Fertilisation.*

I – Introduction

Legumes are particularly important feed sources for livestock due to their high nutritive value (Porqueddu *et al.*, 2003). They can be sown in pasturelands in order to provide feed for livestock directly or indirectly in various forms (eg. forage, hay, silage) (Gibson, 2009). However, the appropriate choice of the legume forage species will have a significant impact on the success of the pastureland, especially under rainfed conditions in the Mediterranean region.

Trifolium subterraneum L. (*T. subterraneum*) is an annual legume species that grows in all types of soils, with a preference to those of moderate texture, lightly acidic to alkaline and tolerates higher pH values (Rossiter, 1978). It is tolerant to grazing, while it has high feeding value as forage as well as hay and silage (Frame, 2005). However, the seeding rate is an important factor that affects the density, the morphological and productive characteristics of plants, the nutritive value as well as the cost of the seeding (McGuire, 1985; Stefanou, 2015).

Phosphorus (P) fertilization promotes and maintains the production of crops and is an important component in the growth of plants, as its deficiency causes a reduction in the growth rate and the final size of plants (Syers *et al.*, 2008). Low phosphorus (P) availability in many lands is one of the most serious problems worldwide, creating problems both on plant growth and on agricultural productivity (Lynch, 2007). Improving the efficiency of phosphorus (P) fertilizer use for crop growth requires enhanced P acquisition by plants from the soil and enhanced use of P in processes that lead to faster growth and greater allocation of biomass (Veneklaas *et al.*, 2012).

Thus, the objective of the present study was to study the phosphorus use efficiency of *T. subterraneum* at two phenological stages as affected by seeding rate under rainfed conditions.

II – Materials and methods

The research was conducted at the farm of Democritus University of Thrace in Orestiada, northeastern Greece (41°33´N latitude, 26°31´E, 33 m a.s.l.) from February to June 2014. The soil is silty clay with pH 7.5 and P (Olsen) 13.2 mg kg^{-1}. The climate of the study area is classified as Mediterranean type, with mean air temperature of 14°C and average annual rainfall of 507mm (Koutroubas *et al.*, 2012). However, during the experimental period the mean air temperature was 14.2°C and the rainfall was 66 mm (Stefanou, 2015).

T. subterraneum cv Geraldton was seeded in 36 plots of $4m^2$ each. The sowing was performed in autumn 2013 but the extremely cold winter resulted in the very low survival rate of *Trifolium subterraneum*. Thus, it was repeated in February 2014. Three seeding rates were tested: 20 kg ha^{-1}, 17 kg ha^{-1} and 13 kg ha^{-1} with six replications per seeding rate. Two levels of fertilization (40 kg ha^{-1} phosphorus and control) were applied before the seeding.

T. subterraneum samples were collected at two phenological stages, early inflorescence in May and fruiting in June. The above-ground biomass production in each plot was determined by using, two 25 x 25 cm quadrats. Plant material was clipped at ground level and placed in individual paper bags. All samples were oven dried at 60°C for 48 h and weighed. Agronomic Phosphorus Use Efficiency (PUE) was calculated by:

$$PUE = \frac{Yhigh - Ylow}{DPapp}$$

were Y_{high} is yield on a high P/fertilized soil, Y_{low} is yield on a low P/unfertilized soil and DP_{app} is difference in amount of P applied as fertilizer between high and low P treatments (Hammond *et al.,* 2009). The results reported on g DM g^{-1} Pf (DM=dry matter; Pf=fertilizer P).

General linear models procedure (SPSS® 18 for Windows) was used for ANOVA. The LSD at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

III – Results and discussion

Phosphorus fertilizer application resulted in a negative PUE (Table 1) at both phenological stages (across seeding rates) and at all the tested seeding rates (across phenological stages) indicating that P fertilization depressed the yield of *T. subterraneum*. However, this depression was more intense at the fruiting stage in comparison to the inflorescence one. PUE was significantly lower at the 17 kg ha^{-1} compared to the seeding rates of 20 kg ha^{-1} and 13 kg ha^{-1}, while there were no significant differences between them. The negative response of *T. subterraneum* to P fertilization can be related to the moderate to high availability of phosphorus in the specific soil, as the optimum level of P (Olsen) in the soil for *T. subterraneum* is 15-20 mg kg^{-1} (McLaren *et al.*, 2015). Moreover, the differences among the seeding rates can be attributed to differences in weeds invasion. Indeed, Katsinikas *et al.* (2015) reported that the production of weeds was significantly higher at the seeding rate of 17 kg ha^{-1} in comparison to the other two seeding rates. It is well known that fertilization operated satisfactorily by weeds, thus increasing their competitive ability and decrease the yield of cultivated plants (Eleftherochorinos, 2002).

Table 1. Phosphorus Use Efficiency (g DM g-1 Pf) of *Trifolium subterraneum* at different phenological stages and seeding rates

Effect	PUE (g DM g^{-1} Pf)
Phenological stage	
Inflorescence stage	-0,41$_a$
Fruiting stage	-6,20$_b$
Seeding rate	
20 kg ha^{-1}	-0,29$_a$
17 kg ha^{-1}	-8,24$_b$
13 kg ha^{-1}	-1,38$_a$

*Means of each treatment followed by the same letter in the column are not significantly different (P≥0.05).

A significant interaction between the phenological stage and the seeding rate was recorded (Fig. 1). Phosphorus fertilization at the seeding rate 13 kg ha^{-1} was more efficient at inflorescence stage, while at the seeding rate 20 kg ha^{-1} was more efficient at the fruiting stage. These were the only cases that P fertilization increased the yield of *T. subterraneum*. However, PUE did not significantly differ between the inflorescence and fruiting stage at the seeding rate of 20 kg ha^{-1}. As no significant differences have been recorded among the seeding rates regarding the dry matter production of unfertilized plants in the inflorescence phenological stage (Stefanou *et al.*, 2016), P fertilization can be efficient and improve forage yield only at this low seeding rate.

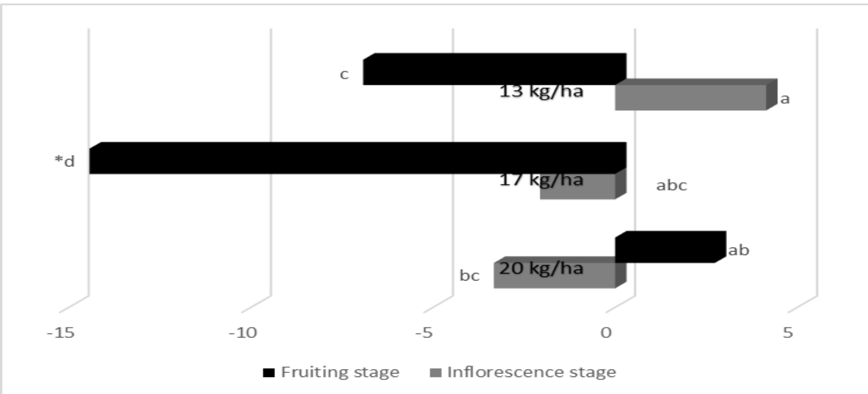

Fig.1. Phosphorus Use Efficiency (g DM g^{-1} Pf) of *Trifolium subterraneum* as affected by phenological stage and seed rate. *Means followed by the same letter are not significantly different (P≥0.05).

IV – Conclusions

Phosphorus fertilizer application had a negative PUE at both phenological stages and at all the tested seeding rates with the exception of inflorescence at the seeding rate 13 kg ha^{-1} and fruiting at the seeding rate 20 kg ha^{-1}. This weak or even negative response of *T. subterraneum* to P fertilization indicates that fertilization is not necessary in soils with moderate to high available phosphorus. Phosphorus fertilization can be applied only in swards established by lower seeding rates that are used for grazing in inflorescence phenological stage.

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