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Effect of different times and techniques of molybdenum application on chickpea (*Cicer arietinum*) growth and yield

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Abstract

Molybdenum (Mo) plays an important role in increasing chickpea yield. In 2009, we studied the effects of different Mo application times and techniques on the response of the *Kabuli* chickpea (*Cicer arietinum*) to Mo nutrition. Pot experiments were conducted under natural conditions using two different soils from northwest Spain and following a factorial statistical pattern (7 × 2) with six replicates for each soil. A treatment of 2 mg Mo pot^{-1} was added to the pots at six different moments, 1 to 6 weeks after emergence (WAE), using two different application techniques (soil and foliar). Both growth and yield were affected by Mo application, but yield was more affected than total dry matter. The response was greater in the medium acid soil than in the neutral-slightly basic soil. At maturity, plants fertilized at 4 WAE produced a greater seed yield, mainly due to an increase in the number of pods per plant. Foliar Mo application was more effective, and soil Mo application should be carried out earlier in the cycle. An interaction was found between time and technique of Mo application, with the highest yield being obtained when Mo was applied at 4 WAE using foliar fertilization.

Additional key words: dry matter; foliar and soil application; *Kabuli* type chickpea; pot experiments; yield components.

Resumen

Efecto de diferentes épocas y técnicas de aplicación de molibdeno sobre el crecimiento y rendimiento del garbanzo

El molibdeno (Mo) juega un papel importante en el incremento del rendimiento del garbanzo. Se estudió durante 2009 la respuesta del garbanzo (tipo *Kabuli*) cultivado en macetas al aire libre a las aplicaciones de Mo en diferentes épocas y utilizando dos técnicas diferentes de aplicación, usando dos suelos distintos del noroeste de España según un diseño factorial (7 × 2) con seis repeticiones para cada suelo. Se añadieron 2 mg Mo maceta^{-1} en seis épocas diferentes, desde la primera semana después de la emergencia hasta la sexta, utilizando dos técnicas de aplicación distintas (al suelo y foliar). El crecimiento y el rendimiento se vieron afectados por la aplicación de Mo, siendo el rendimiento más afectado que la materia seca total. La respuesta fue mayor en el suelo medianamente ácido que en el suelo neutro-ligeramente básico. En la madurez, las plantas fertilizadas con Mo a las 4 semanas después de la emergencia dieron un rendimiento mayor, principalmente debido a un aumento en el número de vainas por la planta. El aumento fue mayor en el suelo medianamente ácido. La aplicación foliar de Mo fue más eficaz; mientras que la aplicación de Mo al suelo debe llevarse a cabo en épocas más tempranas. Se encontró interacción entre la época y la técnica de aplicación de Mo, obteniéndose el rendimiento más alto cuando el Mo fue aplicado vía foliar a las 4 semanas después de la emergencia.

Palabras clave adicionales: aplicación foliar y al suelo; componentes del rendimiento; ensayo en macetas; garbanzo tipo *Kabuli*; materia seca.

Introduction

Chickpea (*Cicer arietinum* L.) is the principal grain legume crop grown in the Mediterranean region, and Spain is a major European chickpea-producing country. Despite its importance, few studies have been carried out on the application of micronutrients to chickpeas. Nevertheless, widespread mineral nutrient deficiencies in soils, together with a low moisture supply, are considered the major environmental stresses responsible for chickpea yield loss (Khan, 1998).

The lack of a nutritional element in a cultivated plant inevitably leads to a decrease in yield. Molybdenum (Mo) plays an important role in increasing chickpea

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Abbreviations used: DW (dry weight); HI (harvest index); WAE (weeks after emergence).

yield through its effects on the plant itself and on the nitrogen-fixing symbiotic process because Mo is directly involved in N fixation by legumes (Roy *et al.,* 2006). In general, it is estimated that each tonne of chickpea grain removes 1.5 g Mo from the soil (Ahlawat *et al*., 2007). Total Mo content of soil varies from 0.2 to 5.0 mg kg^{-1} (Sims, 2000) but Mo in soil is largely unavailable, with less than 0.2 mg kg^{-1} usually being reported as soluble Mo (Sillanpää, 1972). According to Ankerman and Large (1974), soils have low Mo availability when Mo soil content is below 0.11 mg kg^{-1} (ammonium acid oxalate). Mo availability is low in acidic soils, and increases as soil pH approaches neutrality or goes higher (Gupta, 1997; Sims, 2000; Ahlawat *et al.,* 2007). Consequently, Mo deficiency is common in very acid soils, especially with crops that are very sensitive to low concentrations, such as legumes (Sims, 2000). High phosphate levels are positively correlated with Mo deficiency (Ankerman and Large, 1974). Liming may induce micronutrient deficiencies, since it decreases uptake of all micronutrients except Mo (Fageria *et al*., 1997; Roy *et al*., 2006). Mo deficiency produces identical symptoms to nitrogen deficiency (Ahlawat, 1990), but on new leaves. In Mo-deficient chickpeas, the flowers produced are fewer in number, smaller in size and many of them fail to open or mature, leading to lower seed yield (Ahlawat *et al*., 2007). Mo is directly involved in N fixation by legumes (Roy *et al.,* 2006).

Fertilization is one of the growth techniques aimed at increasing unit area yield. Foliar fertilization, soil application and seed treatment are effective application practices with some micronutrients (Ali *et al*., 2000; Deo and Kothari, 2002; Roy *et al*., 2006; Valenciano *et al*., 2010). According to Valenciano *et al*. (2010; 2011) foliar Mo applications 30 days after emergence improved chickpea total dry matter and yield.

This study was conducted in order to determine the effects of different Mo application times and techniques and their possible interactions on growth and seed yield in chickpea.

Material and methods

The experiment was carried out in the Province of León (North-West Spain) in 2009, using two soil types and a small-seeded *Kabuli* chickpea ecotype (cv. Pedrosillano) sown in April and harvested in August. The experiments were carried out following a bi-factorial statistical pattern with six replicates following a randomized block design for each soil type. The first factor was time of application, with seven levels: no application, 1 week after emergence (WAE), 2 WAE, 3 WAE, 4 WAE, 5 WAE, and 6 WAE. The 6 WAE treatment was applied 1-2 days before initiation of flowering. Mo was applied only once on a stated time. The second factor was application technique, with two levels: soil application and foliar application. For each treatment, the amount of Mo applied was 2 mg Mo per pot (solution volume applied per pot, 0.005 L). Mo was applied as a 6.3% (w/v) Mo solution (ammonium molybdate and sodium molybdate).

Plants were grown in PVC pots under natural environmental conditions at Ribas de la Valduerna, Province of León, Spain (42º18.5'N, 5º57.1'W). Pots (210 mm in diameter by 300 mm in depth) were filled with 4 kg of soil. The soils were collected from sites located in Ribas de la Valduerna which had not been fertilized (Gadaña and Trascasas). The main physical and chem-

Table 1. Main physical and chemical characteristics of soils (local name)

	Gadaña	**Trascasas**
Texture (Bouyoucos densimeter)	Loam	Loam
Organic matter (Walkley-Black) (g kg^{-1})	2.1	3.8
pH (1:2.5, water)	5.6	7.2
Electrical conductivity (1:5, water) (dS m^{-1})	0.12	0.16
Calcium carbonate (Bernard calcimeter) (g kg^{-1})	Negligible	Negligible
P (Olsen) (mg kg^{-1})	21.6	9.79
K (1 N NH_4Ac) (mg kg^{-1})	86.0	437.9
Ca (1 N NH_4Ac) (mg kg^{-1})	831.7	2186.4
Mg (1 N NH_4Ac) (mg kg^{-1})	83.9	278.5
Na (1 N NH_4Ac) (mg kg^{-1})	6.9	29.9
Mo (nitric acid digestion) (mg kg^{-1})	2.32	1.43

ical properties of the soils are listed in Table 1. The pH values of the soils were 5.6 and 7.2. Average temperature, average maximum temperature and average minimum temperature during crop growth were 18.4ºC, 24.1ºC and 8.9ºC, respectively. Ten seeds per pot were sown at 3 cm depth on the 23rd of April, 2009. One week after emergence and before starting Mo application, the seedlings were cleared to two plants per pot.

Soil moisture in all pots was maintained at near field capacity by watering plants daily with de-ionized water. Chlorothalonil (tetrachloroisophthalonitrile) and quinosol (8-hydroxyquinoline sulphate) were used to reduce incidence of disease and for chickpea plant protection (Ondategui, 1996). No instances of pests or diseases were observed.

At maturity (13th August), all plants were harvested. Roots, stems with leaves (leaf-stems) and pods including seeds were separated, oven-dried at 80ºC to a constant weight and weighed. The dry weight (DW) data were used to calculate harvest index (HI = seed DW/total DW). Plant yield and yield components (the number of pods per plant, the number of seeds per pod and the 1000-seed weight) were also recorded at harvest. Grain yield (g plant^{-1}) was calculated from the yield components (Grain yield = pods per plant × seeds per pod × 1000-seed weight/1000).

The data were analysed by analyses of variance using SPSS version 15.0.1. software (Steel and Torrie, 1986).

Results

Chickpeas responded to Mo applications, but this response varied according to the soil (Table 2). Significant differences for total DW were only observed for the Gadaña soil, and the applications which yielded greatest total DW were those carried out at 4 WAE. This increased DW was primarily due to greater pod bearing. Meanwhile, significant DW differences in the Trascasas soil were only observed for pod DW, and the highest pod DW was obtained at 5 WAE. Mo application time had an influence on dry matter partitioning between plant organs, whereas Mo application technique had no effect. A relationship between time of Mo application and HI was observed (Figure 1); HI increased until 4 WAE, although the response was very low ($p \leq 0.20$). Furthermore, this response was usually greater for foliar Mo application, thus the relationship was more marked for foliar Mo applications than for soil Mo applications. HI decreased with late Mo applications. Mo foliar application produced more growth than Mo soil application.

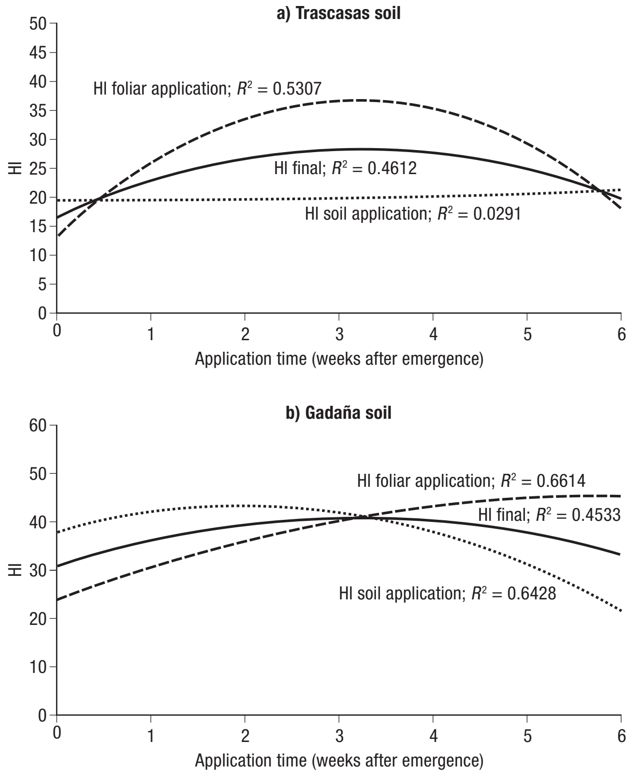

Figure 1. Relationships between Mo application time and Harvest Index (HI) in the two soils.

Yield characteristics were affected by Mo application (Table 2), although response was lower in the Trascasas soil. Different application times produced differences in pods plant^{-1} and in yield: in the Gadaña soil, these differences were highly significant whilst in the Trascasas soil, they were lowly significant. For seeds pod^{-1}, differences were only observed in the Gadaña soil. The highest number of pods plant^{-1} and the highest yields were obtained with treatment at 4 WAE.

Application technique produced differences for yield components and for yield; foliar Mo application resulted in a lower number of seeds per pod but in a higher number of pods per plant and a higher yield.

An interaction was observed between application time and application technique for pods per plant and yield in both soils, and for seeds per pod and for total DW in the Gadaña soil (Table 2). The highest number of pods per plant and the highest yield were obtained with foliar Mo application × 4 WAE (Figures 2 and 3). In the Gadaña soil, the highest number of seeds per pod was obtained with soil Mo application × 6 WAE (Figure 2) and the

Table 2. Dry matter (DW) production at maturity, mean yield components and seed yield of the main treatment with an indication of significance and their coefficient of variation (CV) according to analysis of variance in experiment soils

	Root DW (g plant^{-1})	Leaf-stem DW (g plant^{-1})	Pod DW (g plant^{-1})	Total DW[a] (g plant^{-1})	Yield components			Yield (g plant^{-1})
					Pods plant^{-1}	Seeds pod^{-1}	1000-seed weight (g)	
Trascasas soil								
Application time (T)	NS[b]	NS	$p \leq 0.05$	NS	$p \leq 0.10$	NS	NS	$p \leq 0.10$
No application	0.57	6.16	2.24	8.97	5.50	1.24	295.96	1.87
1 WAE[c]	0.56	5.30	1.63	7.49	4.14	1.23	248.24	1.36
2 WAE	0.56	6.16	2.36	9.08	6.92	1.17	246.52	1.92
3 WAE	0.49	4.00	2.96	7.45	7.58	1.26	260.90	2.48
4 WAE	0.59	4.83	3.28	8.70	8.75	1.19	257.41	2.74
5 WAE	0.61	4.68	3.59	8.89	7.54	1.15	296.17	2.49
6 WAE	0.65	6.15	1.65	8.45	4.00	1.22	302.14	1.38
Application technique (M)	NS	NS	$p \leq 0.05$	NS	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.01$
Soil application	0.56	5.30	2.11	7.97	5.10	1.28	246.76	1.61
Foliar application	0.60	5.36	2.94	8.90	7.60	1.14	298.19	2.45
Interactions: T × M	NS	NS	$p \leq 0.05$	NS	$p \leq 0.05$	NS	NS	$p \leq 0.05$
CV (%)	33.6	33.7	49.8	24.9	49.9	8.9	18.8	49.3
Gadaña soil								
Application time (T)	NS	$p \leq 0.10$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	NS	$p \leq 0.01$
No application	0.38	1.90	1.07	3.35	3.08	1.00	301.49	0.92
1 WAE	0.48	1.85	1.82	4.16	4.25	1.02	334.73	1.47
2 WAE	0.34	1.65	1.92	3.90	4.67	1.12	297.74	1.54
3 WAE	0.31	1.35	2.08	3.74	4.75	1.11	308.86	1.71
4 WAE	0.42	2.41	2.72	5.55	6.50	1.14	315.66	2.32
5 WAE	0.34	1.48	1.13	2.95	2.92	1.00	325.65	0.93
6 WAE	0.41	1.42	1.42	3.26	3.08	1.29	309.87	1.18
Application technique (M)	NS	$p \leq 0.10$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.05$	NS	$p \leq 0.05$
Soil application	0.39	1.55	1.50	3.44	3.57	1.13	314.26	1.26
Foliar application	0.38	1.89	1.97	4.25	4.79	1.06	312.26	1.62
Interactions: T × M	$p \leq 0.10$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	NS	$p \leq 0.01$
CV (%)	38.1	50.6	51.9	43.0	48.7	11.4	7.1	51.6

[a] DW: dry matter. [b] NS: not significant. [c] WAE: weeks after emergence.

highest total DW per plant was obtained with foliar Mo application × 4 WAE (Figure 3). Foliar Mo application gave the highest number of pods per plant and the highest yield at 4 WAE, whereas soil Mo application only improved the number of pods per plant and yield in the Gadaña soil, at 1 WAE.

Discussion

The experiments were conducted using two soils high in total Mo according to Gupta (1997); one acidic soil and another neutral-slightly basic soil. Symptoms of Mo deficiencies (Roy *et al*., 2006) were not observed in any pot. Although chickpeas responded to the soil Mo applications, this response varied with soil (Table 2). One possible cause of a different response could be soil pH, since Mo availability is highly influenced by this factor (Sillanpää, 1972; Gupta, 1997; Sims, 2000; Ahlawat *et al*., 2007). Total DW was higher in the Trascasas soil (8.45 g plant^{-1}) than in the Gadaña soil (3.84 g plant^{-1}). According to Ondategui (1996) and Ahlawat *et al*. (2007), the chickpea grows best in a pH range of 6 to 9, and this could explain the inferior growth observed in the Gadaña soil. The chickpea's low adaptation to acidic soils could also explain why the Gadaña soil produced a smaller yield than the Trascasas soil (1.44 g plant^{-1} and 2.09 g plant^{-1}, respectively). This demonstrates the strong influence of soil types on chickpea performance, which has also been reported by Singh and Sandhu (2006).

Growth was affected by Mo application (Table 2), especially in the acidic Gadaña soil, where plants fertilized at 4 WAE had a greater total dry matter production at maturity, whilst fertilization at 5 WAE, at 6 WAE and no application produced a lower total DW. The 5 WAE and 6 WAE treatments were carried out very next at the initiation of flowering. This increased dry matter production was primarily due to greater pod bearing, followed by leaf-stem DW. Mo application increased growth but only when this was carried out up to 4 WAE. Bhanavase and Patil (1994) obtained DW yield increments with Mo applications carried out 30 days after emergence. Application time of Mo did not greatly influence dry matter partitioning between plant organs. HI increased until 4 WAE (43.52), but the response was very low ($p \leq 0.20$). Foliar Mo application produced more growth than Mo soil application. This increased dry matter production was primarily due to greater pod bearing, followed by leaf-stem DW. In the neutral-slightly basic Trascasas soil, significant differences were only observed for pod DW, and Mo application increased pod DW until 5 WAE. Application time of Mo influenced dry matter partitioning between plant organs. There was not relationship between Mo application time and the relative dry matter production of different chickpea plant organs. Mo application technique did not influence total DW.

On acidic soils, where Mo availability is low, Mo application produces a highly significant improvement in growth whereas on neutral-slightly basic soil, this improvement does not occur. Thus, growth improves with Mo application only where its availability is low. Foliar Mo application is more effective (Fageria *et al*., 2009), since it acts earlier. Foliar applications are also more effective in correcting deficiencies (Ali *et al*., 2000). Moreover, although soil Mo application increases total Mo, Mo availability does not increase as

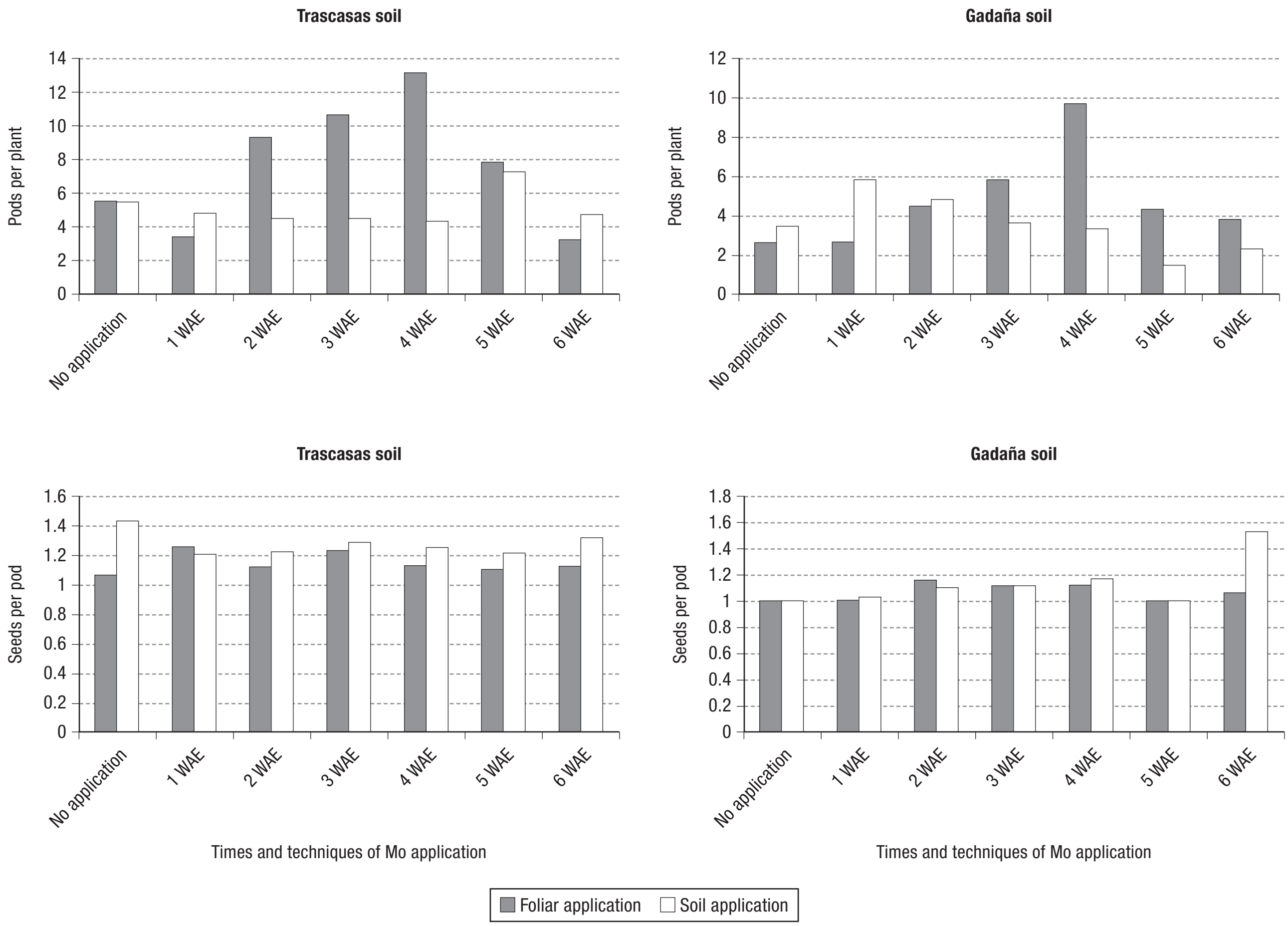

Figure 2. Interaction between application times (WAE: weeks after emergence) and application techniques on pods per plant and on seeds per pod for the two soils.

much as when soil pH is acid. HI was enhanced when Mo was applied between 3 and 4 WAE (Figure 1).

Yield characteristics were affected by Mo application (Table 2), although response was lower on neutral-slightly basic soil (Shil *et al*., 2007). Different application times produced differences in pods plant^{-1} and in yield. Shil *et al*. (2007) also found that yield and pods plant^{-1} were the most influenced by different Mo application levels. In the Gadaña soil, differences were highly significant, whilst in the Trascasas soil these were lowly significant. For seeds pod^{-1}, differences were only observed in the Gadaña soil. The highest number of pods plant^{-1} was obtained at 4 WAE. Furthermore, the highest yield was also obtained with treatment at 4 WAE. Yield was increased due to greater pods plant^{-1} number, this yield component being closely related to final seed yield (Maiti and Wesche-Ebeling, 2001; Valenciano *et al*., 2009). Mo application after emergence improved yield until 4 WAE. Ali and Mishra (2001) gave foliar Mo applications around this time (60 days after sowing) and increased the grain yield significantly. In addition, Bhanavase and Patil (1994) obtained yield increments with Mo applications carried out 30 days after emergence. Braga and Vieira (1998) did not find an increase in yield with late Mo applications (57-60 days after emergence). There were differences for yield components and for yield according to application technique. Foliar Mo application produced a higher number of pods per plant but a lower number of seeds per pod, perhaps due to increased competition between components. Foliar Mo application improved yield and was more effective, since plants absorbed it more quickly even when there was availability in soil. Contrary results were found by Silveira *et al*. (1996) for the common bean (*Phaseolus vulgaris* L.) under field conditions, where soil Mo ap-

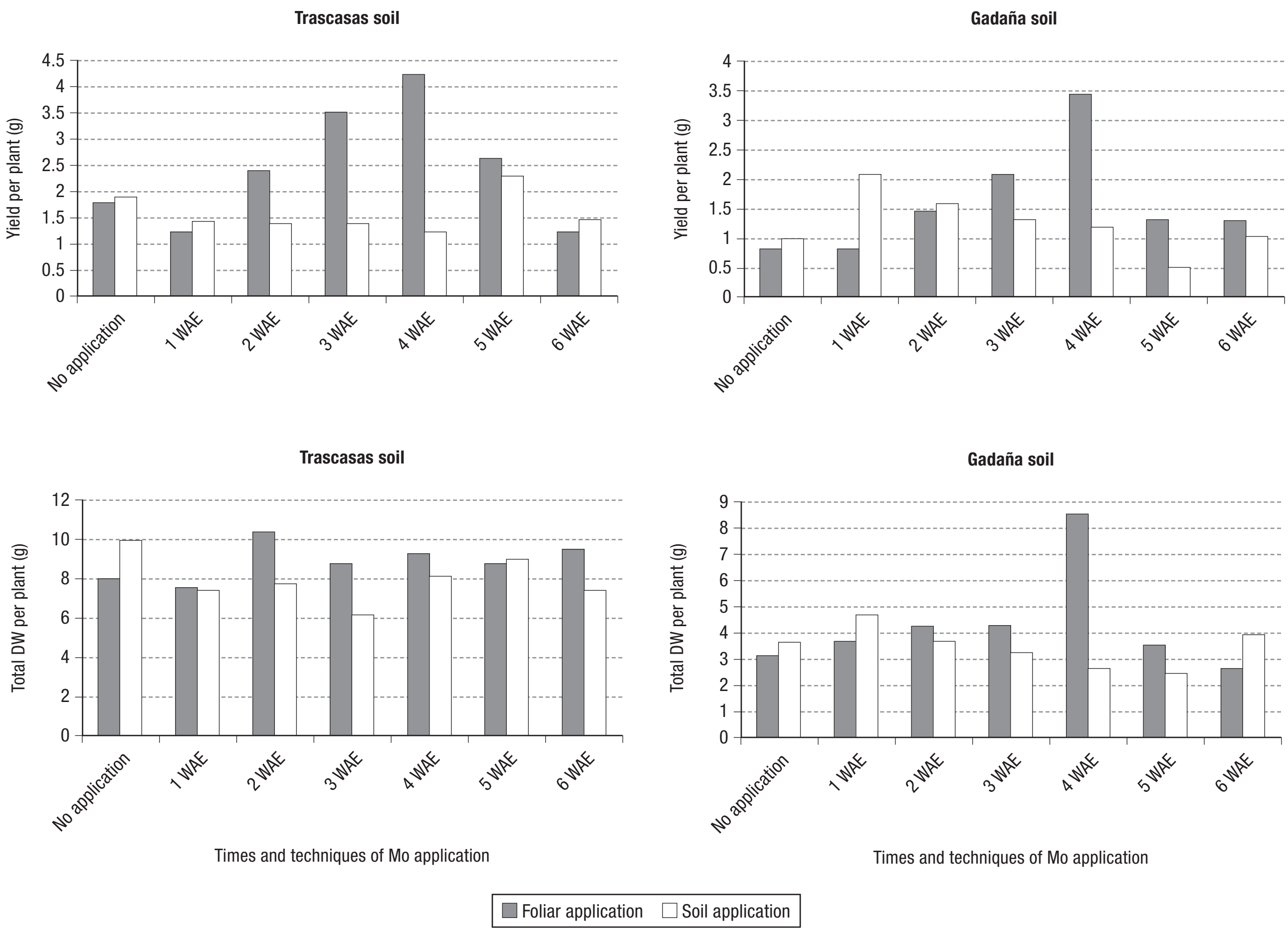

Figure 3. Interaction between application times (WAE: weeks after emergence) and application techniques on yield and total dry weight (DW) for the two soils.

plication at sowing affected grain yield but foliar Mo application 34 days after sowing had no effect.

An interaction was observed between application time and application technique for pods per plant and yield. For foliar Mo application, the highest number of pods per plant and the highest yield occurred when application was carried out at 4 WAE. Soil Mo application only improved the number of pods per plant and yield in the Gadaña soil, and the highest number of pods per plant and the highest yield were obtained when the application was made at 1 WAE. Under these conditions, and in agreement with other studies (Fageria *et al*., 2009), foliar Mo application was more effective for yield increase. The best time for foliar application was at 4 WAE, since a later application could not improve yield. In other leguminous plants, such as the common bean, the best time for foliar application is slightly earlier (Berger *et al*., 1996). According to the results, soil Mo application produced a lower yield increase and was only effective in the acidic soil, where yield was increased when soil Mo application was carried out early (1 WAE). Consequently, soil Mo application must be carried out earlier than foliar application.

As conclusion, this study shows that foliar Mo application was more effective than soil Mo application, under pot conditions at high moisture availability. Mo application increases seed yield due to an increase in the number of pods per plant, principally. Soil Mo application should be carried out earlier in the cycle.

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