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# EFFECT OF LOW TEMPERATURE ON THE NITROGEN NUTRITION OF ANNUAL MEDICS: PRELIMINARY RESULTS

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## ABSTRACT

The "Medics" (annual *Medicago)* developed for the Australian ley-farming system under fairly mild winter temperature are not adapted to most of the agroecological zones encountered in North Africa and West Asia. To allow sheep to graze during winter in place where there is temperature below 10°, was studied the ability of various medics to germinate, fix nitrogen and produce herbage around 10°C. 16 accessions of *Medicago aculeata, M. rigidula, M. tornata, M. truncatula, M. polymorpha, M. rotata* were tested for germination. A following study on seven *M. aculeata* accessions showed that *M. aculeata* appears to be the most permissive accession for early germination and growth at low temperature but requires an efficient rhizobial strain. An experiment in controlled environment conducted to test 5 strains of *Rhizobium meliloti* on four accessions of *M. aculeata* confirmed these results and indicated an interaction between rhizobium and accession of *M. aculeata* for early nodulation.

On a field experiment including inoculated, not inoculated and not inoculated with nitrogen fertilizer treatments, N-fertilized plants assimilated soil nitrate and grew during winter, so at the end of the winter the dry weight of N-fertilized plants was two times the weight of the inoculated or control plants. There was no nitrogen fixation during the cold period; this activity began later during spring. At harvest, herbage yields of N-fertilized and plants inoculated were similar and significantly higher than the uninoculated ones. These results suggest that an early planting enables the medic plants to establish a maximum of plant biomass and active nodulation before winter when photosynthesis and nodulation continues. *M. aculeata* is highly specific for *Rhizobium meliloti*. Selection of *Medicago* and *Rhizobium* for early nodulation and nitrogen fixation is necessary.

**Key words:** *Medicago*, medics, nodulation, nitrogen fixation, nodulation, frost tolerance, *Medicago aculeata*, *Medicago rigidula*.

## INTRODUCTION

The annual medics are mediterranean pasture legumes developed for the Australian ley-farming system under fairly mild winter temperature prevailing in Southern and Western Australia (Puckridge and French 1983).

Australian "know-how" and experience filtered to North Africa (Christinansen *et al.* 1993) especially in various experience in Tunisia (Doolette 1976), Algeria (Carter 1974, Abdelguerfi 1993, Maatougui 1993), Morocco (Bounejmate 1990, Christiansen and Boulanouar 1993), and Libya (Allen 1979, Halse 1993). The same trend occurred in West Asia in Jordan (El-Turk 1993), Syria (Cocks *et al.* 1993), Turkey (Erkan and Yilmaz 1993), Iran (Nazari-Dalshlibrown 1993) and Iraq (Halse 1985).

The introduction of the ley-farming system in North Africa and West Asia was based upon the use of australian commercial cultivars (*Medicago scutellata* cvs Robinson, Sava, Sair,

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*M. truncatula* cvs Jemalong, Cyprus Borung and Ghor, *M. littoralis* cv Harbinger, *M. polymorpha* cvs Circle valley, Serena, *M. rugosa* cv Paragosa, etc., Barnard 1972, Mackay 1982). The poor acceptance of the ley-farming in North Africa and West Asia based on annual medics pastures had certainly to do with the local socio-economics (Nordblom *et al.* 1994) but also with the appropriateness of the cultivars used.

If the availability of locally adapted cultivars of wheat and barley was not a problem in WANA, it was soon clear that Medic cultivars available on a commercial basis from Australia were not adapted to most of the agroecological zones encountered in North Africa and West Asia. The Australian cultivars were selected for the mild to cool winter environment prevailing in the mediterranean austral environment where frost remained exceptional (Gintzburger 1994, Fig. 1). These commercial cultivars proved to be ecologically well adapted to the low lands of North Africa (Coastal zone of Morocco, Tunisia and Libya) while most of the High Plateau of North Africa and West Asia have a noticeable occurrence of freezing days and lower winter temperatures. Ecological distribution of annual medics in WANA (Cocks and Ehrman 1987, Abdelguerfi et Hakimi 1988) and agronomical studies confirmed their frost susceptibility (Prosperi et al, 1991; Prosperi 1993) and hence the need for medics with different biological characteristics from the Australian cultivars (Cocks 1988). Under harsh winter conditions, most of the Australian cultivars faced frost damage and displayed little growth in winter. ICARDA identified potential medic species for replacement of Australian cultivars (Cocks and Ehrman 1987) and initiated numerous medic and *Rhizobium meliloti* germplasm collection (Reid *et al.* 1993, Materon 1993) from cold winter regions of WANA. *Medicago aculeata* and *M. orbicularis* for North Africa and *M. rigidula*, *M. noeana* and *M. rotata* for West Asia appeared to be strong candidates for the cold mediterranean countries of WANA. At ICARDA and for Syria, *M. rigidula* presented the most suitable ideotype (Abdelmoneim and Cocks 1986). For the high plateau of North Africa, *Medicago aculeata* was identified as the best alternative to australian commercial cultivars (Maatougui, pers. com.). This lead ICARDA to launch specific studies in developing new Medics ands their associate rhizobium for the farming system of cold regions of WANA.

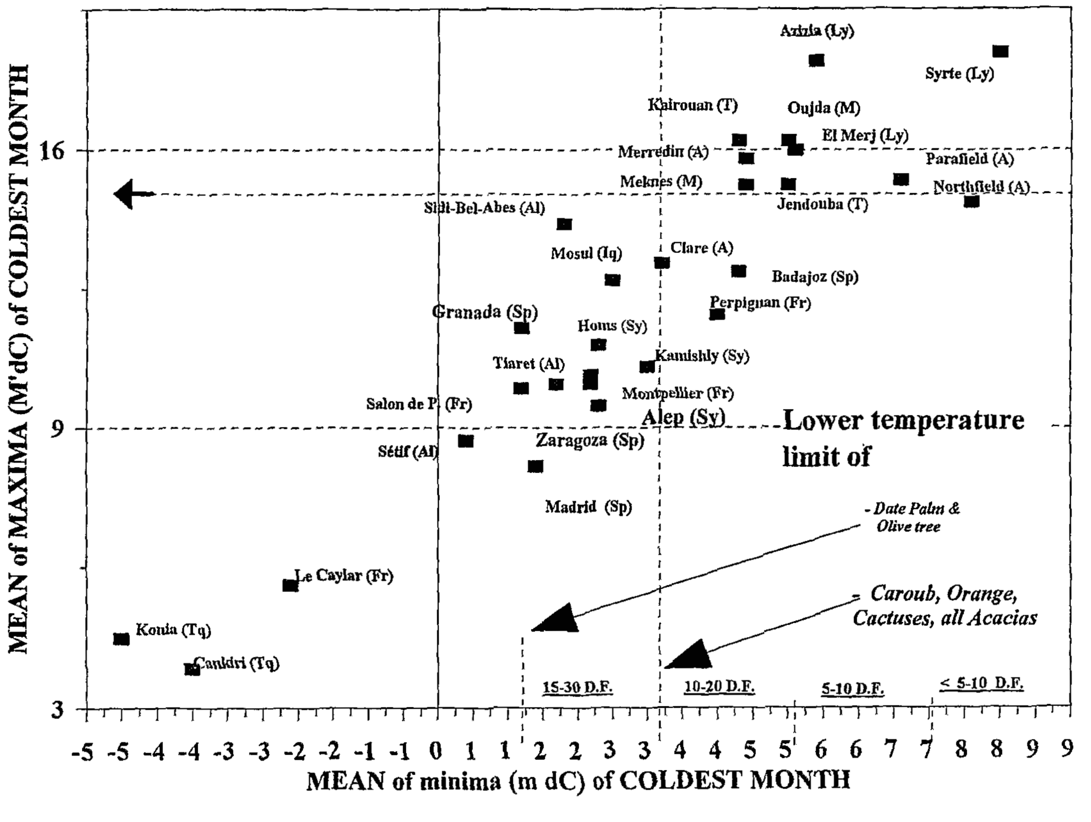



Figure 1. Distribution of evaluation and introduction sites of Medics in WANA in relation to mean minimum and maxima temperature of the coldest month (Gintzburger 1994).

Achieving these objective may allow sheep to graze for longer period during winter after an early germination soon after the early autumn rainfall. Therefore, the purpose of this study was to find medics capable of growing early and fixing nitrogen at low temperature.

It is generally admitted that temperate legumes nodulate and fix $N_2$ in a temperature range 10-30°C and the tropical ones in the temperature range 15-35°C. The temperature range for functioning the symbiosis is narrower than that of the plant supplied with combined nitrogen. The influence of high temperature on symbiosis is well documented. There are differences between strains as shown by Lie *et al.*(1976) on Pea. There is less information about the influence of cold on nodulation and nitrogen fixation. It is known that rhizobium strains isolated from cold soils of Northern Europe are more efficient for nodulation at low temperature than those from temperate soils (Allen *et al*, 1964; Ek-Jander et Fahreus, 1971). The lower limit for the symbiosis is also dependent of the variety of the host plant but different species of *Medicago* were not screened for this capacity. Nodule initiation is particularly sensitive to low temperature, but nodules formed at a favorable temperature maintain nitrogen fixation when transferred to lower temperature, even 2°C. (Dart and Day, 1971). The nitrogenase enzyme in the nodules is less affected by low temperature than the formation of bacteroids in the internal nodule tissue. For sub-clover (*Trifolium subterraneum*), at 7°C, almost no bacteroid tissue can be observed; the highest amount of bacteroid tissue was formed between 11° and 15°C., but the efficiency of nitrogen fixation per unit of bacteroid tissue and the plant dry weight was the greatest at 19°C. (Roughley, 1970). On some legumes, at least, nitrogen fixation is relatively insensitive to low root temperature but nodulation is delayed (Lynch and Smith, 1993).

## EXPERIMENT

We studied the ability of various medics to germinate, grow, fix nitrogen and produce herbage at low temperature, around 10°C. A part of this study was made on 16 to 19 species or accessions of *Medicago aculeat* , *M. rigidula*, *M. tornata*, *M. truncatula*, *M. polymorpha, M. rotata*. Further, we decided to concentrate on *M. aculeata* and to studied the behaviour of 7 accessions originated from Morocco, Algeria, Syria and Jordan.

The comparison of the behaviour of these 16 to 19 species or accessions at low temperature showed that *M. rigidula* acc. 1871 germinate quickly at 8°C (Fig. 2a) but do not grow well in the field in winter and produce a moderate amount of dry weight during the spring (Fig. 2b and 2c).

*M. aculeata* acc. 5099 germinate quickly at low temperature (Fig. 2a), similarly to *M. aculeata* 3006 and much better than *M. aculeata* acc. 2917, 5897 or 3058. For the growth during winter (Fig. 2b), acc. 5897, 3058 and 5099 performed well but not acc. 3006 and for the final yield (Fig. 2c) acc. 2917 produce much more dry weight than acc. 5099 and 3006 because these two last did not find efficient strains of rhizobium in the soil or in the mixture of strains used in the inoculum. In conclusion, *M. aculeata* acc. 5099 appears to be the best choice if we use efficient strains for this very specific medic.

In a growth chamber adaptable to low temperature (Conviron), we studied the ability to nodulate at 10°C for 4 accessions of *M. aculeata* and 5 strains of *R. meliloti* (Table 1 give the result for 4 accessions and 5 strains). These preliminary results showed that, for *Medicago aculeata*, 50% of the plants nodulated at optimum temperature in 9.4 days after inoculation, and at 10°C in 21,3 days. There is a significant strain-accession interaction for 50% germination at 10°C, for example, the *Medicago acculeata* acc. 80 nodulate in 25.6 days with strain M527 and in 16,6 days with strain M538. This experiment also confirmed the field trial showing that *M. aculeata* acc. 5099 is very specific.

A screening for the efficiency of *Rhizobium meliloti* on accessions 80 and 5099 was undertaken in a greenhouse. This experiment showed a strong interaction between strains and accessions (Fig. 3a and 3b). The most efficient strains for accession 80 was M527, M538, M53, M619 and M375 but these strains were not efficient at all on accession 5099. Only strains M620 and M567 were efficient on both accessions in these conditions.

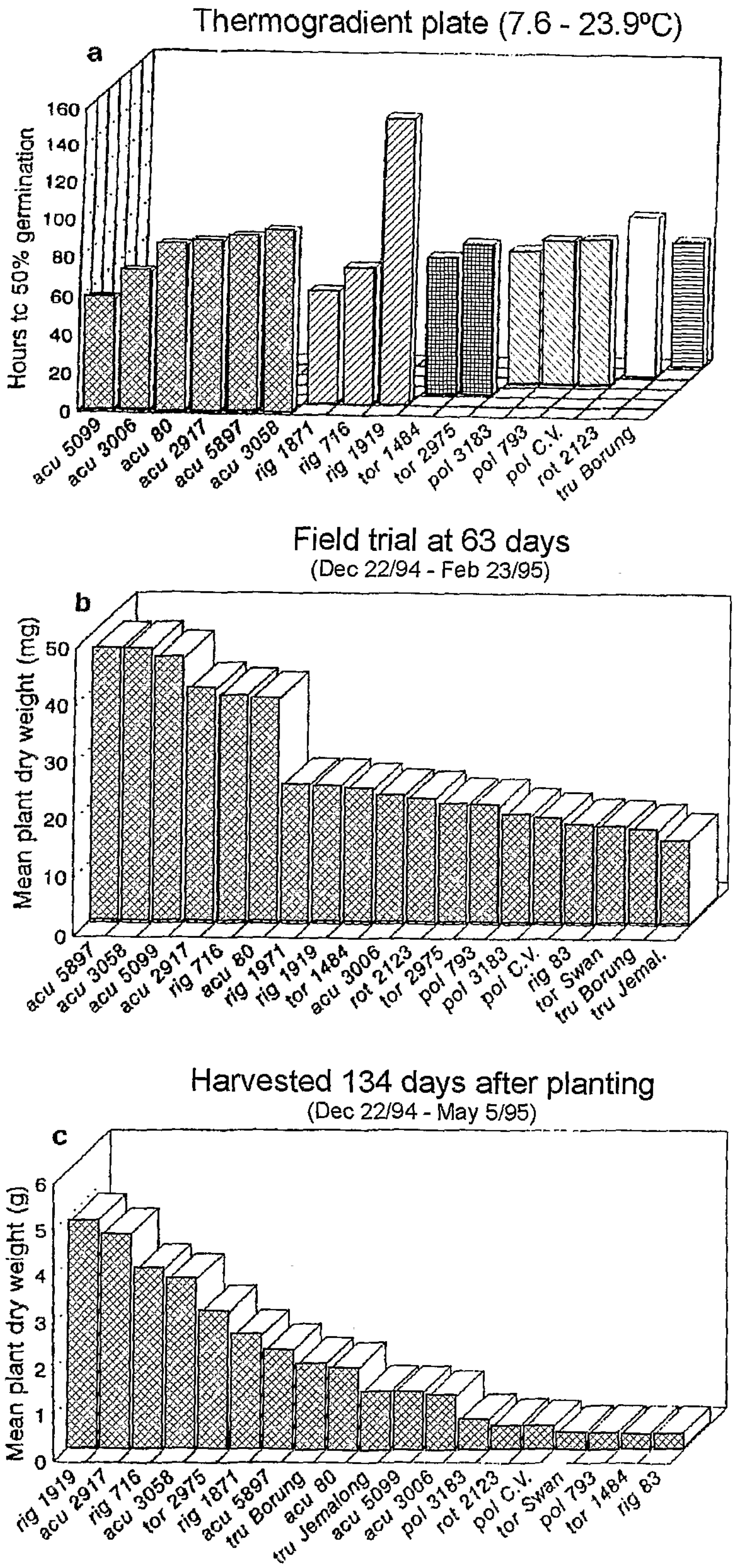



Figure 2. Influences of cold temperature on 16 to 19 accessions of medics. (a) Time in hours to obtain 50% of germinated seeds on a thermogradiant plate at 8°C (16 accessions). (b) Dry weight of the aerial parts of 19 accessions of medics at the end of the winter, intergrating ability to germinate and grow at low temperature before that any nitrogen fixation occurs (sowing date December 22, 1994). (c) Dry weight of the aerial parts of 19 accessions of medics at harvest ( May 5, 1995).The difference in the classification with Fig. 2b is mainly due to the presence or not of efficient nodules.

Table 1. Number of days for 50% of plant nodulated at (+) optimum temperature (15° night 22° day) and at (-) low temperature (10°) for four accessions of *Medicago aculeata* and five strains of *Rhizobium meliloti*. Monoxenic culture of the plants in ²test tube on nitrogen free agar medium (Vincent, 1971)

| Accession of *M. aculeata* | Temperature | Strain of *meliloti* M29 | M53 | M375 | M527 | M538 |
|---|---|---|---|---|---|---|
| 80 | + | - | - | - | - | - |
| | - | 22.6 | 22.8 | 19.2 | 25.6 | 16.6 |
| 3058 | + | 11.2 | 9.8 | 9.2 | No nod | 11.6 |
| | - | 19.6 | 22.2 | 19.2 | No nod | 21.0 |
| 5099 | + | ← No nodulation → | | | | |
| | - | ← No nodulation → | | | | |
| 5897 | + | 7.0 | 8.2 | 7.6 | 8.6 | 11.2 |
| | - | 24.2 | 19.2 | 21.0 | 24.4 | 20.8 |

No nod: no nodulation
- not measured

<u>Origin of the strains of *Rhizobium meliloti*</u>, all belonging to the ICARDA, PFLP collection. M29: Zerbeh, Syrie; M53 Sweida, Syria; M375, Marrakech, Morocco; M527, Aksehir, Turquey (1071 m); M538, Ilgen, Turkey (1030m )

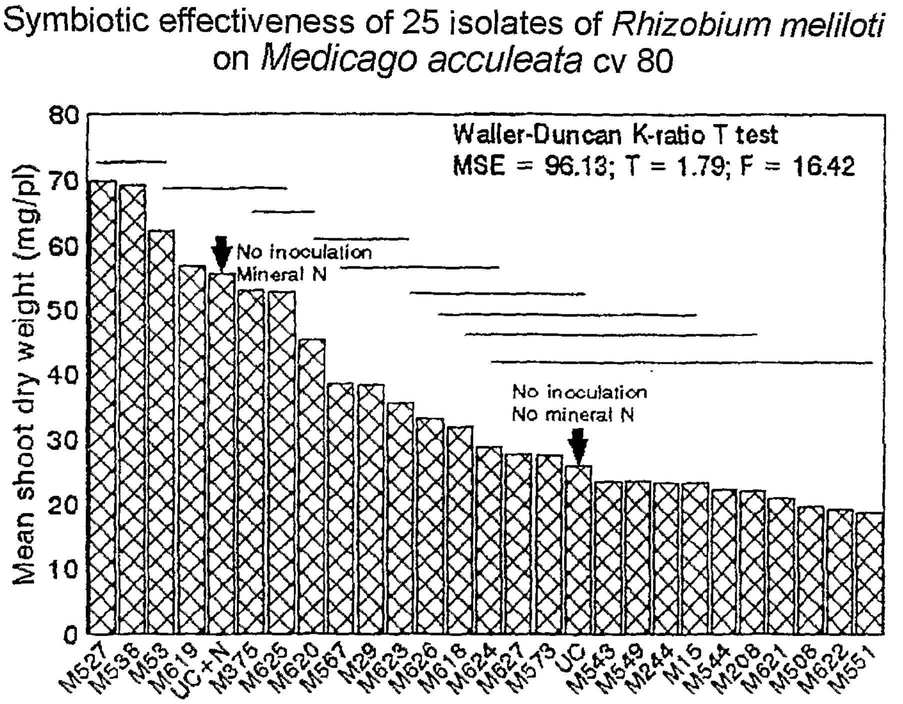
<u>Origin of the seeds of *Medicago aculeata*</u> given by the Genetic Resources Unit from ICARDA acc. 80 IDGC, Algeria; acc. 3058, Settat, Morocco; acc. 5099 Tafila, Jordan; acc. 5897, El Mehir, Algeria.

On december 22, 1994, we established a field trial at Tel Hadya (ICARDA-Aleppo) to observe the behaviour of *Medicago aculeata* during the winter. We used the accession 80 with 4 treatments, not inoculated with or without nitrogen (30 kg ha$^{-1}$ N-urea at sowing and 30 kg at flowering) and inoculated even with strain M53, or with strain M29.

During winter, at soil temperature, at 7° to 10°C at 5 cm. depth (Fig. 4) strain M53 nodulated quickly, 28 days after sowing (on January 18) but nitrogen fixation began only after winter, 64 days after sowing (on February 23) and after that increased quickly (Fig. 4c). Therefore, nodules were present but did not develop a bacteroidal tissue and did not fix. Nevertheless, this medic was able to grow in winter because, in the plots fertilized with N (Fig. 4b), the dry weight of the plant at the end of the winter (March 14, 83 days after sowing) was two times higher than for the nodulated plants (Fig.4c). That can be due to the fact that the maximum air temperature (Fig.4a) reached 15° to 20°C almost each day during winter. Therefore leaves were at higher temperature than nodules, at least for some hours each day. At harvest, the herbage dry weight was 1.36 t/ha (± 0.41 t) for the uninoculated control, equivalent to 1.14 t/ha (± 0.37 t) for the plot inoculated with the strain M29 (which is not efficient on this accession) and two times higher for the plots fertilized with N (2.59 t/ha ± 0.51 t) and for the plots inoculated with strain M53 (2.43 t/ha ± 0.51 t). In this soil, where the *Rhizobium meliloti* were present but not efficient on this very specific medic, the efficient strain M53 used in the inoculum dominated the native strains. This treatment multiplied the yield by two and gave a yield equivalent to a fertilization with 60 kg/ha of nitrogen.

## DISCUSSION AND CONCLUSIONS

These preliminary results have to be repeated another year with a new set of seeds. Nevertheless they confirm the possibility to screen medics and probably also rhizobium for growth and nitrogen fixation at low temperature. *Medicago aculeata* and likely some *M. rigidula* perform well in these conditions. *M. aculeata* accession 5099 germinate, grow and nodulate early in winter and is probably the most promising for the production of herbage at low temperature, but inoculation with an efficient strain of *Rhizobium meliloti* is necessary for this ecotype.



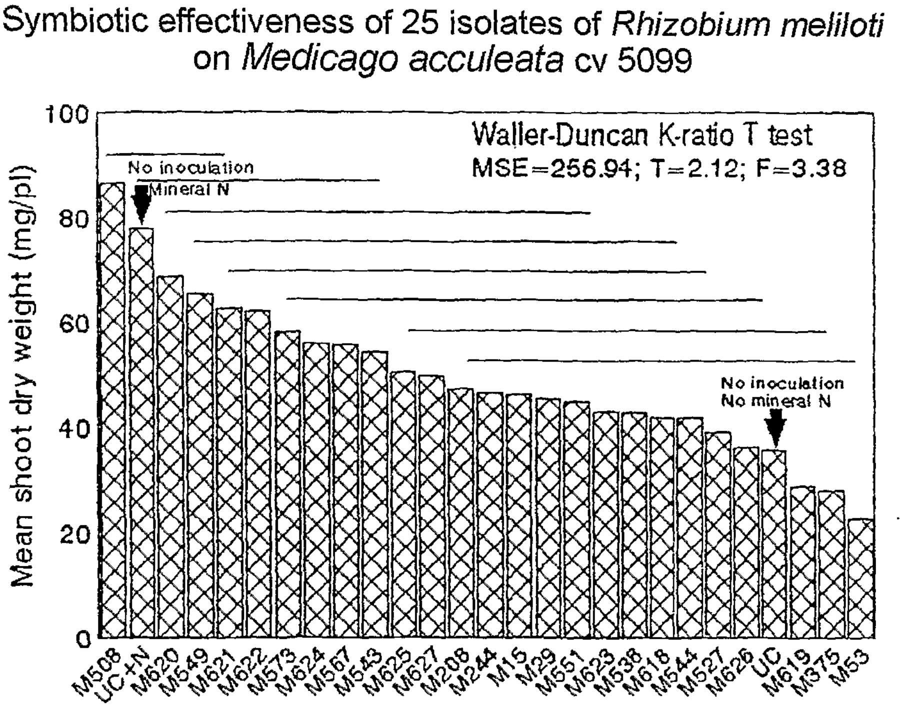




Figure 3. Efficiency test for 25 strains of *Rhizobium meliloti* on two accessions of *Medicago aculeata.* The inoculated seeds were grown on sterile vermiculite and nitrogen-free solution. with two controls, one with no inoculation and another one with no inoculation but with addition of mineral N in the solution. In these conditions, the dry weight of the inoculated plants were representative of the efficiency of the strain to fix $N_2$. Dry weight per plant (average of 6 replicates).

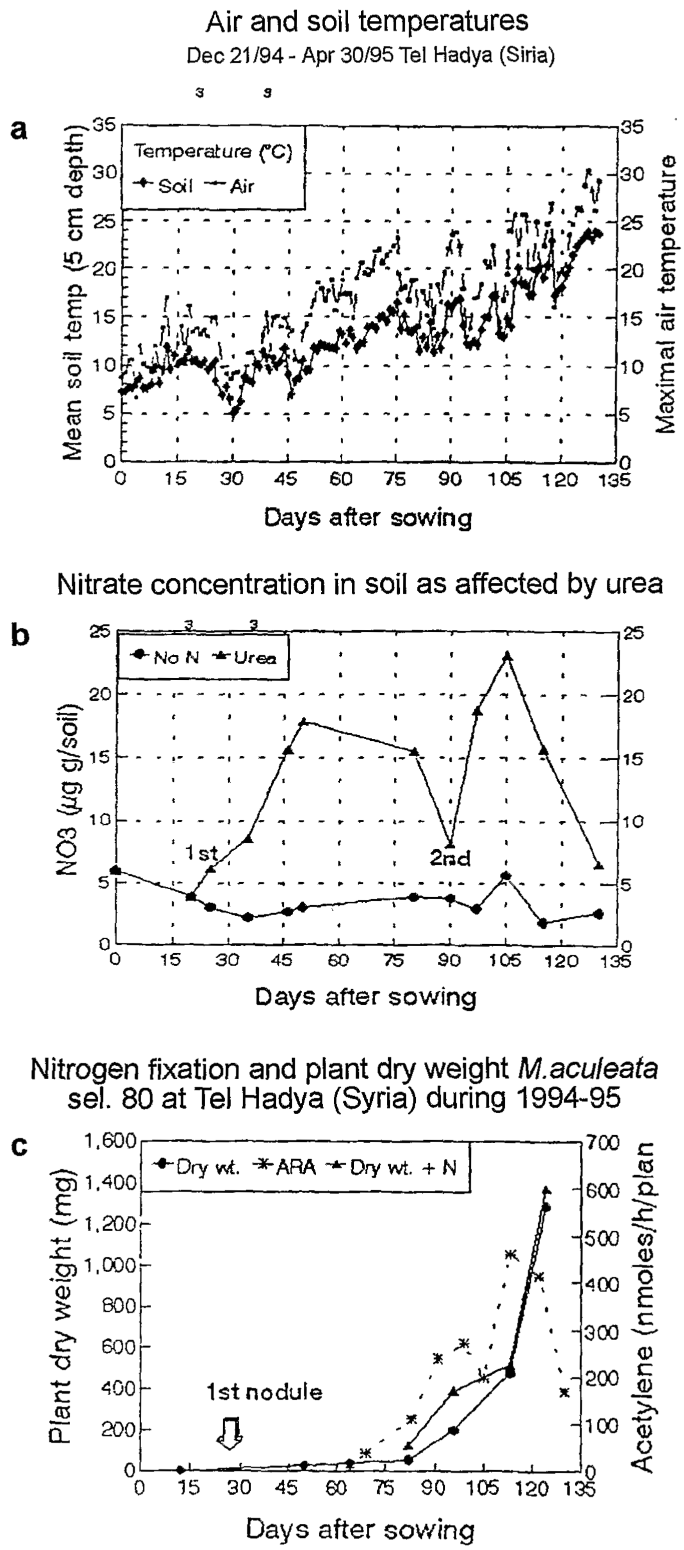



Figure 4. Field experiment with *Medicago aculeata* accession 80, sown at Tel Hadya (Syria) on december 22, 1995. (a) Average soil temperature ( -5 cm), and maximum air temperature. (b) $NO_3^-$ level in the soil (in ppm $NO_3^-$ in dry soil with or without application of urea). 1st and 2nd application correspond each time at 30 kg N-urea. (c) Dry weight of aerial parts of the plant for the treatments "inoculated with strain M 53" and "not inoculated but with addition of 60 Kg N". Nitrogenase activity (-----x-----) measured by the *in situ* technic using acetylen reduction (Balandreau and Ducerf, 1978).

Nodules were formed in the field at low temperature during winter but they fixed nitrogen only 36 days later and, during this time, the plants do not grow. During the same period plants fed with urea were able to grow, showing that photosynthesis and nitrate assimilation are working in winter at least during the hottest hours of air temperature (around 15°C, see Fig. 4a).

We may propose two hypothesis to explain this difference on growth of the plants between the two ways of nitrogen nutrition.

1) During winter, the medic has only some leaves and not enough energy is available to induce nitrogen fixation which is more expensive than nitrate assimilation (Salsac and Chaillou, 1981).

2) Nodules can be induce at an average soil temperature of 8° to 10°C but the bacteroidal tissue do not develop and there is no nitrogen fixation. But almost each day during winter, the air and leave temperature reach 15° to 20°C, which allows nitrate assimilation and growth.

To clarify this question, this study will be continued by a thesis of a syrian student working mainly at ICARDA under the direction of a professor of ENSAIA of Nancy and a professor of the Faculty of Agronomy of Aleppo.

These preliminary results showed that the most efficient way for nitrogen nutrition of medics during winter is N fertilizer, but N fertilization is not applicable on rangeland. The solution could be to establish the annual medic pastures in areas where there is no heavy frost, early in the autumn, so that a large photosynthetic apparatus and an active nodulation may develop before winter. Therefore, photosynthesis and nitrogen fixation may continue in winter, during the days of lower temperature (till 3° to 5°C) and the growth of the plant will continue. Our study also showed that there is variability between accessions of medics and probably also between strains of rhizobium for growth and nitrogen fixation at 8°-10°C. The pasture production of medic during winter and early spring can be increased by screening the two partners of the symbiosis.

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