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Effect of Different Soil Temperatures on Three Annual Medics

[1]Reza Tabatabaie, [1]Majid Amini Dehaghi and [3]Shahram Jafari
[1]Department of Agronomy, Faculty of Agriculture, Shahed University, Tehran, Iran
[2]Department of Computer Science and Engineering,
Faculty of Engineering, Shiraz University, Shiraz, Iran

Abstract: Effect of different soil temperature on growth and development of yield and yield components for three annual *Medicago* species was investigated in a control growth chamber. In this experiment, the species of annual medics namely *Medicago polymorpha*, *M. radiata* and *M. rigidula* that suppose to be adapted with cold and temperate zones were used. Root zone temperatures at four levels including 5, 10, 15 and 20°C were considered. The experimental plan was factorial in base of randomized complete block design with four replications. Result showed that the annual *Medicago* species had significant difference for producing dry matter and yield components. The species of *M. polymorpha* and *M. rigidula* had more leaf, stem and root dry matter, plant height, leaf and stem to root ratio, leaf to stem ratio and leaf area and yield than *M. radiata*. Therefore these species may be better for ley-farming system in cold and temperate zones. Also, the result showed that the 5°C root zone temperature had effectively decreased the yield and yield components of the annual medics. In conclusion, application of ley-farming system is not successful in the zone that soil temperature is lower than 5°C especially for vegetative stages. Therefore, in the zones that soil temperature is more than 10°C, annual medics have normal growth and produced suitable yield. Ley-farming system (cereal-legume rotation) could be replaced with fallow-cereal rotation.

Key words: *Medicago radiata*, *M. rigidula*, *M. polymorpha*, Root zone temperature, yield

INTRODUCTION

Ley-farming systems (Including cereal and annual medics) are extensively used in South Australia (Puckridge and French, 1983). During past 20 years, some effort is performed for settlement of similar system in Mediterranean zone, Algeria, Tunisia, Syria, Libya and Iraq. Unfortunately, in spite of ley-farming high potential for agricultural development, the results were not satisfactory.

There are not annual medic cultivars adapted to different environmental conditions and this is the most important drawback stopping us to achieve the goals of planting alfalfa. For example, new Australian cultivars have been used in Algeria (Rapport IDGC, 1980) and Syria (Cocks and Ehrman, 1987) but their cultivation was not successful because they showed sensitivity to coldness.

There are *Medicago radiata* (Cocks and Ehrman, 1987), *Medicago rigidula* and *Medicago noeana* in cold zone of Iran and Turkey (Cocks, 1992). Presence and abundance of annual medics in each environment can indicate how annual medic cultivars adopt to that environment (Cocks and Ehrman, 1987). *Medicago polymorpha* has extensive dispersion therefore this medic should be the first selection.

The minimum and maximum temperatures for growing annual medics are 3-7°C and 30-35°C, respectively. The precipitation for development zone of annual medics is 300-600 mm (Bounejmate *et al*., 1992). Honson (1988) has announced that leaf growth of annual medics stops at lower than 5°C and new leaves firstly increased up to 30°C and then it decreased.

Soil temperature in range of 10-16°C and air temperature in range of 15-30°C is suitable for annual medic germination (Puckrige and French, 1983). Annual air mean temperature for *M. polymorphis* is between 10.5 to 27°C (Duke, 1981). This cultivar is relatively resistant to coldness (Madson, 1951).

Alfalfa (*Medicago sativa* L.) was mentioned in (Franklin *et al*., 2005) to have an interaction between flooding and soil temperature. It was demonstrated that flooding injury increases as temperatures increase. Because root respiration is often reduced under lower temperatures, less injury to flooded plants may occur under cold temperatures simply due to a lower metabolic rate and delay in root mortality.

Benzarti Benz (2004) used Degree-days (dd) to compute an index for the efficiency of the thermal effects of the shelter on well-watered Lucerne, under favorable (10 to 30°C) and unfavorable (above 30°C) temperature

Corresponding Author: Reza Tabatabaie, College of Agriculture, Shahed University, Tehran, Iran P.O Box 14155-4838, Tel: (98)21-55572400-10

conditions. Daytime temperatures were disadvantageous to Lucerne and even with a high degree of water availability, there were no significant differences between sheltered and unsheltered conditions.

The result showed that *M. rigidula* cultivar has the most potential for survival in winter (Krall *et al*., 1996). The other researchers found that *M. rigidula* is more resistant to cold than other annual medics cultivars in very hard winter (Abd El Moneim and Cocks, 1986; Cocks and Ehrman, 1987).

The research about effect of root zone temperature on morphological growth and development, yield and yield component of annual medics are very low; therefore this research is essential for increasing forage production.

MATERIALS AND METHODS

Seeds of three annual medic cultivars (*Medicago polymorpha* cv. santiago, *Medicago radiata* and *Medicago rigidula* cv. rigidula) were surface sterilized in ethanol alcohol with 98% purity and sodium hypochlorite (2% solution containing 4 mL L^{-1}). Then seeds were rinsed thoroughly with distilled water and then planted in trays containing sterilized sand. Nine days old seedlings at two cotyledon initial stages, were transplanted into sterilized 15 cm plastic pots in growth chamber containing the same medium. Each pot had 10 annual medic plants. A 1 cm layer of Perlite was placed on top of the sand in each pot to act as insulation. The growth chamber light (300 μmol m^{-2} s^{-1}) was provided by Cool-white fluorescent tubes. Light intensity across the growth chamber was measured several times during this experiment and was always uniform. The photoperiod was 12 12 h (day: night). As this work examined the influence of Root Zone Temperature (RZT), air temperature was held at 20 15°C day: night. Root zone temperature (±0.2°C) was controlled by circulating cooled water around the big pots with twelve pots settled in each tank. The 12 medium pots containing plants were put into the big pots. A hole was drilled at the bottom of each pot to allow the pots to drain. After being transplanted into the pots the plants were acclimatized for 24 h prior to inoculation. The inoculums were produced by culturing *Rhizobium meliloti* in medium without agar, into 450 mL sterile flasks. The medium into flasks was autoclaved at 121°C in 20 min prior culture. Each inoculated plant received 1 mL of a 4 day old (log phase) culture which was adjusted with distilled water. The inoculums were cooled to the corresponding root zone temperature and applied by pipette to the root area. Plants were watered with a Hoagland's solution (Hoagland and Arnon, 1950). Prior to each watering, the added Hoagland's solution was temperature-adjusted to the treatment root zone temperature.

Experimental design was arranged at Shahed University in 2005. Its experimental plan was in a factorial in base of a randomized complete block design with 4 replications and a total of 4 temperature treatments (5, 10, 15 and 20°C) and three varieties. Plants were harvested at 51 days after inoculation and the following data were collected: Leaf area (Delta-T area meter, Delta-T Devices Ltd., Cambridge, England); Leaf number, Root, Stem and Leaf dry weight, Branch number, Stem node number, Plant height and Root length. Results were handled statistically by analysis of variance using the SAS system (SAS Institute Inc., 1997). When analysis of variance showed significant treatment effects, Duncan's multiple range test was applied to make comparisons among the means at the 0.05 level of significant (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

There are significant differences at 1% level among varieties for plant height. Maximum and minimum heights were for *M. polymorpha* (7.36 cm) and *M. rigidula* (3.63 cm), respectively. Root zone temperature had significant effect on plant height 5 and 20°C RZT treatments had the lowest (3.01 cm) and highest (7.54 cm) plant height, respectively (Table 1 and 2). The result is

Table 1: Means comparison of morphological traits, yield and yield component of different medics annual varieties

Traits	*M. radiata*	*M. polymorpha*	*M. rigidula*
Plant height (cm)	4.63b	7.36a	3.63c
Root length (cm)	27.06a	24.25b	28.97a
Root dry matter (mg)	114.00b	161.00a	136.00ab
Stem dry matter (mg)	37.00b	69.00a	49.00b
Leaf dry matter (mg)	134.00b	154.00a	164.00a
Leaf to stem ratio	3.49a	2.38b	3.71a
Leaf and stem to root ratio	0.795b	0.974a	1.02a
Branch No. per plant	1.61a	0.93b	1.76a
Stem node No.	9.53a	9.1a	8.45b
Leaf No. per plant	14.21a	9.64b	13.88a
Leaf area (cm^2)	27.43b	32.71a	28.56ab

Means with similar letter (s) in each column, are not significantly different at 5% probability level according to Duncan's multiple range test

Table 2: Means comparison of root zone temperature on morphological traits, yield and yield component of different medics annual varieties

Traits	20°C	15°C	10°C	5°C
Plant height (cm)	7.54a	5.24b	5.05b	3.01c
Root length (cm)	28.78b	32.93a	31.88a	13.45c
Root dry matter (mg)	82.00c	249.00a	140.00b	78.00c
Stem dry matter (mg)	59.00b	81.00a	39.00c	28.00c
Leaf dry matter (mg)	191.00a	210.00a	120.00b	83.00c
Leaf to stem ratio	3.49a	2.94a	3.16a	3.02a
Leaf and stem to root ratio	3.27a	1.23b	1.52b	1.53b
Branch No. per plant	1.55b	2.44a	1.04c	0.71c
Stem node No.	9.31b	10.16a	9.59ab	7.05c
Leaf No. per plant	13.9b	17.60a	10.40c	8.20b
Leaf area (cm^2)	47.91a	40.50b	21.58b	8.28b

Means with similar letter (s) in each column, are not significantly different at 5% probability level according to Duncan's Multiple Range Test

Table 3: Correlation coefficient among traits of medic annual varieties in different root zone temperature

Traits	Leaf area	Stem node No.	Branch No.	Leaf No.	Leaf and stem to root ratio	Leaf and stem ratio	Leaf dry matter	Stem dry matter	Root dry matter	Root length
Stem node No.	0.49**	1								
Branch No.	0.43**	0.48**	1							
Leaf No.	0.57**	0.66**	0.90**	1						
Leaf and stem to root ratio	0.39**	-0.14ns	-0.08ns	-0.06ns	1					
Leaf to stem ratio	0.03ns	0.04ns	0.38**	0.37**	0.15ns	1				
Leaf dry matter	0.84**	0.56**	0.67**	0.77**	0.15ns	0.07ns	1			
Stem dry matter	0.61**	0.36**	0.31*	0.37**	0.04ns	-0.51**	0.75**	1		
Root dry matter	0.31*	0.46**	0.43**	0.49**	-0.63**	-0.22ns	0.56**	0.60**	1	
Root length	0.58**	0.60**	0.35**	0.62**	-0.07ns	0.23ns	0.66**	0.37**	0.57**	1
Plant height	0.69**	0.36**	-0.09ns	0.03ns	0.40**	-0.37**	0.45**	0.56**	0.09ns	0.21ns

* and ** significant at 5 and 1% probability levels, respectively. ns: Not significant

Table 4: Interaction effects of annual medic varieties and root zone temperature on plant height (cm)

Root zone temperature (°C)	*M. polymorpha*	*M. radiata*	*M. rigidula*
5	3.10	3.60	2.25
10	7.42	3.90	2.9
15	8.20	4.87	3.4
20	10.60	6.07	5.9

Table 5: Interaction effects of annual medic varieties and root zone temperature on root length (cm)

Root zone temperature (°C)	*M. polymorpha*	*M. radiata*	*M. rigidula*
5	13.1	14.5	12.7
10	32.2	26.8	34.8
15	28.8	35.1	36.6
20	22.8	31.8	31.6

similar to Zhang *et al*. (1995) that is about effect of low root zone temperature on Soya plant growth. Interaction effect between varieties and root zone temperature on plant height had significant differences at 1% level. There are positive correlations at 1% level between plant height and stem and leaf dry matter, leaf area, stem node number and leaf and stem to root ratio (Table 3). Comparison of the means showed that the maximum and minimum plant height were for *M. polymorph* at 20°C RZT (10.6 cm) and *M. rigidula* (2.25 cm) at 5°C RZT, respectively (Table 4).

There are significant differences between varieties for root length at 1% level. *M. rigidula* (28.97 cm) and *M. polymorpha* (24.25 cm) had maximum and minimum root length, respectively. RZT had significant effect on root length all varieties had the lowest (13.45 cm) and highest (32.93 cm) root length at 5 and 15°C RZT, respectively (Table 1 and 2). The result is similar to bean (*Vicia faba*) research that is about increasing meristem cells size under increasing RZT to 25°C (Evans and Savage, 1959). Carwford and Huxter (1977) showed that temperature accretion from 2 to 14°C in RZT cause root development interaction among pea varieties (*Pisum sativum*). Interaction effects among varieties and RZT had significant differences for root length at 1% level. Mean comparisons showed that the maximum and minimum root length was belongs to *M. rigidula* under 15 (36.6 cm) and 5°C (12.7 cm), respectively (Table 5). Root length had positive correlation at 1% level with root, stem and leaf dry matter, leaf number, leaf area, stem nod number and branch number (Table 3).

Table 6: Interaction effects of annual medic varieties and root zone temperature on root dry matter (mg)

Root zone temperature (°C)	*M. polymorpha*	*M. radiata*	*M. rigidula*
5	110	64	62
10	202	40	177
15	258	245	243
20	74	108	64

The varieties had significant differences with each other for root dry matter. *M. polymorgha* (161 mg) and *M. radiata* (114 mg) had the maximum and minimum root dry matter, respectively. RZT had very significant differences on root dry matter. The 15 and 5°C RZT produced the lowest (78 mg) and highest (249 mg) root dry matters, respectively (Table 1 and 2). The result is similar to research of Walsh and Layzell (1986). They observed that low RZT decreased root dry matter of Soya. Interaction effect between varieties and RZT was significant for root dry matter. Mean comparisons is showed that *M. polymorph* (258 mg) and *M. radiata* (40 mg) had the highest and lowest root dry matter at 15 and 10°C, respectively (Table 6). Root dry matter had positive correlation with root length, stem and leaf dry matter, leaf number, stem node number and branch number at 1% level (Table 3).

There are significant differences among varieties for stem dry matter. *M. polymorpha* (69 mg) and *M. radiata* (37 mg) had the highest and lowest stem dry matter, respectively. The RZT had significant effect on stem dry matter. The highest and lowest stem dry matter was obtained under 5 (28 mg) and 15°C (81 mg) RZT, respectively (Table 1 and 2). Low RZT decreased shoot dry matter of Soya (Zhang *et al*., 1995). Interaction effect between varieties and root zone temperature was significant for stem dry matter. Mean comparisons were showed that *M. polymorpha* and *M. radiata* had the most and least stem dry matter under 15 (106 mg) and 5°C (21 mg) RZT, respectively (Table 7). Positive correlation

Table 7: Interaction effects of annual medic varieties and root zone temperature on stem dry matter (mg)

Root zone temperature (°C)	*M polymorpha*	*M radiata*	*M. rigidula*
5	33	21	26
10	57	24	40
15	106	59	78
20	80	44	54

Table 8: Interaction effects of annual medic varieties and root zone temperature on leaf dry matter (mg)

Root zone temperature (°C)	*M polymorpha*	*M radiata*	*M. rigidula*
5	93	66	90
10	147	67	146
15	183	215	232
20	194	190	188

between stem dry matter and plant height, root length, leaf and stem dry matter, leaf number, leaf area, stem node number and branch number were observed at 1% level (Table 3).

Leaf dry matter of cultivars was different. *M. rigidula* cultivar with 164 mg and *M. radiata* cultivar with 134 mg had the highest and lowest leaf dry matter. Root zone temperature had significant effect on leaf dry matter. So, the plants in 15°C (210 mg) and 5°C (83 mg) temperature produced the maximum and minimum leaf dry matter, respectively (Table 1 and 2). Soya researches showed that root zone temperature decreased plant weight, leaf number and area. This result is similar to ours (Zhang *et al*., 1995). Interaction effects between cultivars and root zone temperature were significant for leaf dry matter. Mean comparisons showed that *M. rigidula* cultivar at 15°C and *M. radiata* at 5°C root zone temperature had the most (232 mg) and the least (66 mg) leaf dry matter (Table 8). Leaf dry matter had positive correlation with plant height, root length, root and stem dry matter, leaf number, stem node number and branch number at 1% level (Table 3).

Cultivars were different for leaf to stem ratio. *M. rigidula* cultivar and *M. polymorpha* had the most (3.71) and the least (2.38) leaf to stem ratio, respectively. Root zone temperature had not significant effect on leaf to stem ratio (Table 1 and 2). Cultivar and root zone temperature interaction had significant effect on leaf to stem ratio. Mean comparison showed that the maximum and minimum leaf to stem ratio was related to *M. radiata* cultivar (4.32) at 20°C and *M. polymorph* cultivar (1.75) at 15°C, respectively (Table 9). Correlation between leaf to stem ratio with leaf number and branch number were positive at 1% level (Table 3).

Cultivars were different for leaf and stem to root ratio. *M. rigidula* cultivar and *M. radiata* had the most (1.02) and the least (0.795) leaf and stem to root ratio, respectively. Root zone temperature had not significant effect on leaf and stem to root ratio (Table 1 and 2). Cultivar and root zone temperature interaction had significant effect on leaf and stem to root ratio. Mean comparison showed that the maximum and minimum leaf and stem to root ratio was related to *M. polymorpha* cultivar (3.72) at 20°C and *M. polymorph* cultivar (1.01) at 10°C, respectively (Table 10). Soya experiments showed that leaf and stem to root ratio increased with raising root zone temperature. This result is the same as ours (Zhang *et al*., 1995). Correlation between leaf and stem to root ratio with leaf area and plant height were positive at 1% level (Table 3).

Table 9: Interaction effects of annual medic varieties and root zone temperature on leaf to stem ratio

Root zone temperature (°C)	*M. polymorpha*	*M. radiata*	*M. rigidula*
5	2.80	2.70	3.54
10	2.57	3.29	3.62
15	1.75	3.68	3.40
20	2.42	4.32	3.70

Table 10: Interaction effects of annual medic varieties and root zone temperature on leaf and stem to root ratio

Root zone temperature (°C)	*M. polymorpha*	*M radiata*	*M. rigidula*
5	1.18	1.51	1.92
10	1.01	2.29	1.25
15	1.21	1.21	1.26
20	3.72	2.23	3.85

Table 11: Interaction effects of annual medic varieties and root zone temperature on leaf number

Root zone temperature (°C)	*M. polymorpha*	*M. radiata*	*M rigidula*
5	6.1	8.7	10.0
10	8.3	8.5	14.5
15	11.9	21.1	19.7
20	12.1	18.4	11.3

Cultivars were different for leaf number and *M. radiata* cultivar and *M. polymorpha* had the most (14.21) and least (9.64) leaf number, respectively. Root zone temperature had significant effect on leaf number. Plants under 15 and 5°C temperature produced the most (17.6) and the least (8.2) leaf number, respectively (Table 1 and 2). Soya experiments showed that leaf number increased with raising root zone temperature. This result is the same as ours (Zhang *et al*., 1995). Interaction effect between cultivars and root zone temperature had significant effect on leaf number of *M. radiata* cultivar under 15°C temperature and *M. polymorpha* under 5°C had the most (21.1) and the least (6.1) leaf number, respectively (Table 11). Leaf number had positive correlation with root length, root, stem and leaf dry matter, leaf to stem ratio, leaf area, branch number and stem node number at 1% level (Table 3).

There are significant differences among varieties for leaf area. *M. polymorpha* and *M. radiata* varieties had the highest (32.71) and the lowest (27.43) leaf area, respectively. Effect of root zone temperature on leaf area was significant the varieties produced the most leaf area (47.91 cm^2) and the least (8.28 cm^2) under 20 and 5°C, respectively (Table 1 and 2). The result is similar to Soya result that showed the plants produced more leaf number

Table 12: Interaction effects of annual medic varieties and root zone temperature on leaf area (cm^2)

Root zone temperature (°C)	*M polymorpha*	*M radiata*	*M rigidula*
5	6.1	8.7	10.0
10	36.0	14.2	14.5
15	38.0	39.5	44.0
20	50.7	47.2	45.7

Table 13: Interaction effects of annual medic varieties and root zone temperature on branch number

Root zone temperature (°C)	*M polymorpha*	*M radiata*	*M rigidula*
5	0.67	0.92	1.52
10	0.17	0.40	1.55
15	1.52	2.97	2.82
20	1.35	2.15	1.15

Table 14: Interaction effects of annual medic varieties and root zone temperature on stem node number

Root zone temperature (°C)	*M polymorpha*	*M radiata*	*M rigidula*
5	7.17	7.82	6.15
10	9.50	8.75	10.70
15	8.97	10.97	10.55
20	10.75	10.77	6.42

in high root zone temperature (Zhang *et al*., 1995). Interaction among varieties and root zone temperature for leaf area was significant. The maximum and minimum leaf areas were observed in *M. polymorpha* under 20°C temperature (50.7 cm^2) and *M. polmorpha* under 5°C temperature (6.1 cm^2), respectively (Table 12). There are positive correlation between leaf area with plant height, root length, dry matter of root, stem and leaf, leaf and stem to root ratio, leaf number, branch number and stem node number at 1% level (Table 3).

Significant differences were observed between varieties for branch number. *M. rigidula* and *M. polymorpha* had the most (1.76) and the least (0.93) branch number, respectively. Root zone temperature affected on branch number. The most and the least branch number were for plants under 15°C (2.44) and 5°C (0.7) RZT, respectively (Table 1 and 2). Varieties and root zone temperature interaction was significant for branch number. *M. radiata* produced the highest branch number (2.97) under 15°C RZT and *M. polymorpha* had the lowest branch number (0.17) under 5°C RZT (Table 13). Branch number had significant positive correlation with root length, leaf and root dry matter, leaf number and leaf area at 1% level (Table 3).

Stem node number was different among varieties. *M. radiata* and *M. rigidula* had the highest (9.53) and lowest (8.45) node number. Root zone temperature had significant effect on stem node number. Plants under 10 (10.16) and 15°C (7.05) RZT produced the most and the least node number, respectively (Table 1 and 2). Significant interaction between varieties and root zone temperature was observed on stem node number. *M. radiata* had the most stem node number under 15°C RZT (10.97) and the least stem node number was observed in *M. rigidula* under 5°C RZT (6.15) (Table 14). Stem node number had significant positive correlation with plant height, root length, stem and leaf dry matter, leaf number, leaf area and branch number at 1% level (Table 3).

CONCLUSIONS

The findings of the experiment clearly stated that the varieties were different for dry matter production and yield component. *M. rigidula* and *M. polymorpha* were the best varieties regarding the leaf, stem and root dry matter, plant height, leaf and stem to root ratio, leaf to stem ratio, leaf area and yield. In conclusion, these varieties are suitable for cultivation in ley-farming system in cold and moderate zone. The next suitable variety is *M. radiata* for cold and moderate zone. This variety produced lower yield than *M. rigidula* and *M. polymorpha*.

Also, 5°C of root zone temperature reduced yield and yield component of annual alfalfa varieties. The results were analogous to other researchers' result that worked on other legumes. Therefore, using ley-farming system is not successful in the zone that soil temperature reaches to five degrees of centigrade or less in growing season; however, annual alfalfa can grow very properly in zones that soil temperature reaches to 10°C or more in growing season. In this zone, traditional rotation system such as fallow-cereal can be replaced by ley-farming system such as crop-pasture.

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