Growth, Nodulation and Nitrogen Fixation Capacity of Egyptian Clover

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Experiments were conducted to evaluate the Egyptian clover *(Trifolium alexandrinum* cv. Miscawi) as a winter forage crop under 3 and 6-week harvesting intervals and its symbiotic nitrogen fixation capacity. Forage yield within each growing period, total seasonal forage yield and seed production showed a significant variation among the harvesting intervals, indicating that potential yield for this crop may not be expressed unless a compatible harvesting interval is imposed. Egyptian clover gave significantly higher forage yield and seed production under the 6-week interval compared with the 3-week harvesting interval.

Symbiotic N fixation was estimated by total N content techinque. Egyptian clover showed an early nodulation and an average crude protein content in the shoot of 18.4%. Average efficiency of N fixation for this crop was 40.35 mg N/gm dry wt./ nodule/ day. It is concluded that Egyptian clover is a dependable winter crop in producing good forage yield with a high nutritive value under a proper management system. Moreover, the potential for N fixation in this crop is sufficient to sustain high yield under a suitable management system. Finally, it is suggested that the effect of seeding date on the performance of this crop and its water soluble carbohydrate trends should be evaluated.

INTRODUCTION

Agricultural development activities in Libya are directed towards diversifying crops as well as annual production. However, the latter created its own needs which led directly to the identification of additional forage crops suitable for cultivation under our conditions, and producing consistently a good yield, which is comparable to, or better than the existing forage crops.

Forages have a limited contribution to the primary food supply of mankind due to their fibrous nature. However, forage crops are grown primarily to provide digestible energy and other nutrients, including protein and minerals for ruminants, that provide an important link for the conversion of forage to human food. Even in countries where ruminants are fed liberal grain supplements to increase milk production and to fatten them, forages provide 63% and 73% of the nutrient requirements for dairy and beef cattle, respectively, and 89% of the feed requirements for sheep and goats (2). In areas where grain feeding is not common, ruminants obtain about 95% of their nutrient requirements from forage (2). Management systems involving harvesting and conservation make it possible to take full advantage of the seasonal input of light energy falling on the crop and so to obtain the maximum return of digestible energy.

Oats have long been used as winter forage crops. However, because of the low income per unit land, and its long growing season to make hay from it, there is a growing recognition that other winter crops have to be tried. Egyptian clover *(Trifolium alexandrinum* L.) has been suggested as a winter forage crop. Moreover, several studies indicated that management systems greatly affect yield of forage crops (1, 5, 10, 11). Therefore, considerations should be given to the development of management systems, which would better utilitze available moisture, more efficiently utilize available solar energy, and eventually increase the productivity of the available land. This study sought to evaluate Egyptian clover as a winter forage crop under different harvesting intervals, and to assess its symbiotic nitrogen fixation capacity.

METHODS AND MATERIALS

I - Cutting Frequency:

This experiment provides a background for assessing potential of different cutting frequencies for improving forage yield and subsequently, animal performance.

Miscawi, Egyptian clover (a multi harvests variety) at 25 kg/ ha was seeded on Oct. 3 and Oct. 16, 1983 and 1984, respectively. The experimental area was fertilized at the rate of 100 kg P_2O_5/ ha at the time of seeding. Plots were seeded in solid rows at 30cm spacing. Plot size was 3 × 3 m. Cutting frequency treatments that were defined with fixed intervals of 3 and 6 weeks between cuts following a common first harvest were in randomized complete block design with 3 replications. The primary objective was to evaluate regrowth, so a common first harvest was used followed by the differential cutting frequencies. Dates for the first common harvest were Dec. 25, 1983 and Jan. 15, 1985. At each harvest Forage yields were taken with a sicklebar plot mover at a height of 10 cm. Dates and number of cuts for each cutting frequency were as follows:

Harvesting Interval				Date		
1983-1984	Jan.	Feb.	Feb.	Mar.	Apr.	Apr.
	16	7	27	20	9	30
3-week	1	2	3	4	5	6
6-week		1		2		3
1984-1985	Feb.	Feb.	Mar.	Apr.	Apr.	May
3-week	5	26	19	9	30	21
	1	2	3	4	5	6
6-week		1		2		3

After determination of the fresh weight of the whole plot, a forage sample from 1 m2 was taken from each harvested plot. Samples were oven dried at 75°C for 24 hours, and forage yield was calculated on an oven dry basis. Since cuts were made every 3 or 4 weeks, the two forage yields of sequential 3-week cuts were added to give data for each 6-week growth interval. This allowed direct comparison of cutting frequencies for each 6-week growth period. The experiment allowed for three growth periods of 6 weeks during 1983-1984 and 1984-1985 growing season. Ending dates were 7 Feb., 9 Apr. and 21 May, 1985. Data for each 6-week growth period comparison were analyzed separately and summed to provide the seasonal forage yield data. Regrowth after the last common harvest was allowed for seed production.

II - Symbiotic Nitrogen Fixation Capacity:

Miscawi Egyptian clover at 25 kg/ ha. was seeded on 15 Nov. 1985 in solid rows at 30 cm spacing. Four replications were used in this experiment. Plot size was 5×10 m^2. The experimental area was fertilized with P at the rate of 100 kg. P_2O_5/ ha.

Strating from 14 Dec. 1985, a 50 cm^2 random sample was taken from each plot weekly. At the time of sampling, plants were digged and pulled carefully from soil, roots were washed free of soil. Plants were then counted and separated into roots, and above-ground vegetative material. Nodules were dissected from the roots. All samples were oven dried for 24 hours at 100 degree C. Dry weight data were expressed on a per plant basis. Different plant parts were ground to pass 40-mesh screen for nitrogen determination by the Kjeldah1 method. Soil nitrogen at seeding was considered to be negligible. For example in 1984 wheat was the previous grown crop. It was given 100 kg N/ha at the time of planting, so little residue was likely following harvesting. The whole experimental area was harvested on 18 Jan., 15 Mar. and 3 May 1986, at 10 cm height. Regrowth after the last harvest was allowed for seed production.

All experiments reported in this paper were conducted at the Facultyof Agriculture. Research Farm on sandy loam soil (with adequate K in the soil). Plots were sprinkler irrigated as needed during the course of the experiments.

RESULTS AND DISCUSSION

No significant difference between harvesting frequencies was observed for forage yield obtained from the first common harvest in either 1983-1984 and 1984-1985 growing season (Table 1). Therefore, changes in forage production on subsequent growth periods were mainly related to harvesting frequency differences and not to a bias in randomization.

Table 1 — Forage yield for the first common harvest of 1984-1985 and 1985-1986 growing seasons.

Harvesting Interval	Growing season 1984-1985	1985-1986
	Ton/ ha	1.27
3 weeks	0.97a*	1.26a
6 weeks	1.12a	1.36a

* Harvesting interval mean within each growing season followed by the same letters are not significantly different ($p < 0.05$).

Differences among harvesting intervals for forage yield of the first, second and third growing period were significant (Table 2). Egyptian clover gave greater yield under the more infrequent harvesting. As harvesting interval increased from 3 to 6 weeks, seasonal forage yield increased about 112%, and 179% in 1983-1984 and 1984-1985 growing season, respectively. These comparisons included the forage yield from the first, second and third growing period. If forage production from the first common harvest, and the fourth growing period was allowed for forage and not for seed pro-

Table 2 — Forage of Egyptian clover for different growth periods, total forage summed over growing period, and seed production under 3 and 6-week harvesting interval at 1983-1984 and 1984-1985.

Harvesting Interval	Forage yield* Growing Period First	Second	Third	Total	Seed
1983-1984		tons/hac			Kg/ha
3-weeks	1.97b	1.63b	2.11b	5.71b	86.37b
6-weeks	2.71a	4.29a	5.12a	12.12a	134.15a
1984-1985					
3-weeks	1.37b	1.03b	2.46b	4.86b	98.97b
6-weeks	2.96a	4.69a	5.93a	13.58a	160.26a

* Harvesting intervals means for each growing season followed by different letters are significantly different ($p < 0.05$).

Table 3 — Nitrogen concentration and content of different plant parts of Egyptian clover.

Samp.1—ling date	Mean N Concentration. Shoot	Root	Nodules	Mean N Content/ Plant Shoot	Root	Shoot + root	Nodule	Total
		%				mg		
1	3.33	2.35	5.98	5.54	1.41	6.95	0.48	7.43
2	3.08	2.45	6.14	7.15	1.98	9.13	0.81	9.94
3	3.19	2.32	5.62	9.78	2.27	12.05	0.97	13.02
4	3.20	2.20	5.73	14.94	3.63	18.57	1.45	20.02
5	3.09	2.15	5.52	25.97	4.74	30.71	1.97	32.68
6	3.06	2.29	5.50	32.53	5.23	37.76	2.45	40.21
7	3.06	2.24	5.98	10.87	5.12	15.99	2.15	18.14
8	3.74	2.73	6.45	16.41	8.01	24.15	2.94	27.09
9	3.37	2.30	4.22	25.95	7.12	33.07	2.95	36.02
10	3.22	2.21	5.39	35.67	10.44	46.11	3.51	49.62
11	3.05	1.97	5.65	48.07	7.68	55.75	3.63	59.38
12	2.76	1.69	5.64	56.80	7.22	64.02	3.89	67.91
13	3.12	2.30	6.20	74.12	9.42	83.54	4.44	87.98
14	2.83	2.27	6.16	93.24	9.89	103.13	4.98	108.11
15	2.09	2.27	5.86	21.98	13.81	35.79	2.90	38.69
16	2.85	2.22	4.99	45.87	10.69	56.56	3.04	59.60
17	2.28	2.15	5.37	63.23	23.25	86.48	3.50	89.98
18	2.87	2.19	4.74	105.24	16.00	121.24	4.05	125.29
19	2.46	2.11	4.77	132.81	21.45	154.26	4.26	158.52
20	2.37	2.21	4.49	165.38	20.88	186.26	5.13	191.39
mean	2.95	2.23	5.52	49.58	9.51	59.09	2.98	62.05

duction, are considered, forage production by Egyptian clover would be higher than it was reported here. Limberg and Enayat (4) reported that, the highest seasonal yield and the best regrowth was obtained by taking the first harvest at the late bud stage (about 40 to 60 days after seeding) and succeeding harvests at 35 to 50 days intervals. Moreover, Nelson *et al* (6) reported that the best yield from this crop was obtained when it was harvested at 1/4 bloom stage.

Table 2 shows that the first growing period gave the lowest yield compared with the other two growing periods. Thus, it appears that the cool temperature of the late fall and winter, was the most limiting factor on forage production and growth of Egyptian clover, especially in early development of this crop. Therefore, it was suggested that the effect of seeding date on forage yield and growth of Egyptian clover should be established.

Seed production under both harvesting intervals is shown in Table 2. When the last regrowth was allowed for seed production, the amount of seeds produced under both harvesting intervals was significantly different (Table 2). Averaged over both growing seasons, the amount of seeds produced was 92.7, and 147.2 kg/ ha, under the 3 and 6-week harvesting interval, respectively.

Harvesting frequency of forage crops has several effects on their productivity (1, 5, 10, 11). The amount of leaf-area remaining and level of water soluble carbohydrate reserve (WSC) determine the nature of their regrowth and plant persistence. Results of this experiment agree with the majority of previous experiments, namely that higher forage yields were produced from infrequent harvesting compared with frequent harvesting (1, 5, 10). The higher forage yield of Egyptian clover obtained with 6-weeks harvesting interval is probably explained partially on the basis that the canopy was allowed to develop to intercept a higher proportion of radiation. Clear differences existed among harvesting intervals.

The growth pattern of this crop is shown in Fig.1. The increase in dry weight of the vegetative material of this crop was almost linear in time. As time progressed, the relative increases in dry weight increased, apparently in response to the better temperature prevailing in spring and early summer. Moreover, rate of growth appeared to be controlled by the supply of introgen from nodules. This was shown by the close relationship between plant dry weight and nitrogen content ($r = 0.91$, $p < 0.05$). Moreover, this acceleration in growth coincided with the time when the nodules reached a reasonable size. In general, growth trend of roots and nodules and the nodulation process was similar to that of the above vegetative material (Fig. 1). Nodules were very small at first, but developed rapidly later on (Fig. 1). These data indicated that nodule weight bore a close relationship to the weight of the whole plant ($r = 0.88$, $p < 0.05$). Moreover, Fig. 1 shows that harvesting greatly restricted development of nodules. Following harvesting nodule weight decreased for a period of about 1 week and then started to increase. This was probably due to an increasing proportion of nodules becoming senescent and/ or mobilization of photosynthates in order to support regrowth, which led to a reduction in supply of energy to nodules. Thus, the data were consistent with the observations of Lawrie and Wheeler (3) that nodules requires a continuing supply of photosynthates.

The trends in whole plant nitrogen concentration and content (Table 3) indicated an earlier and rapid development of nitrogen fixation in Egyptian Clover. The different plant parts maintained a similar trend in N concentration during the course of the ex-

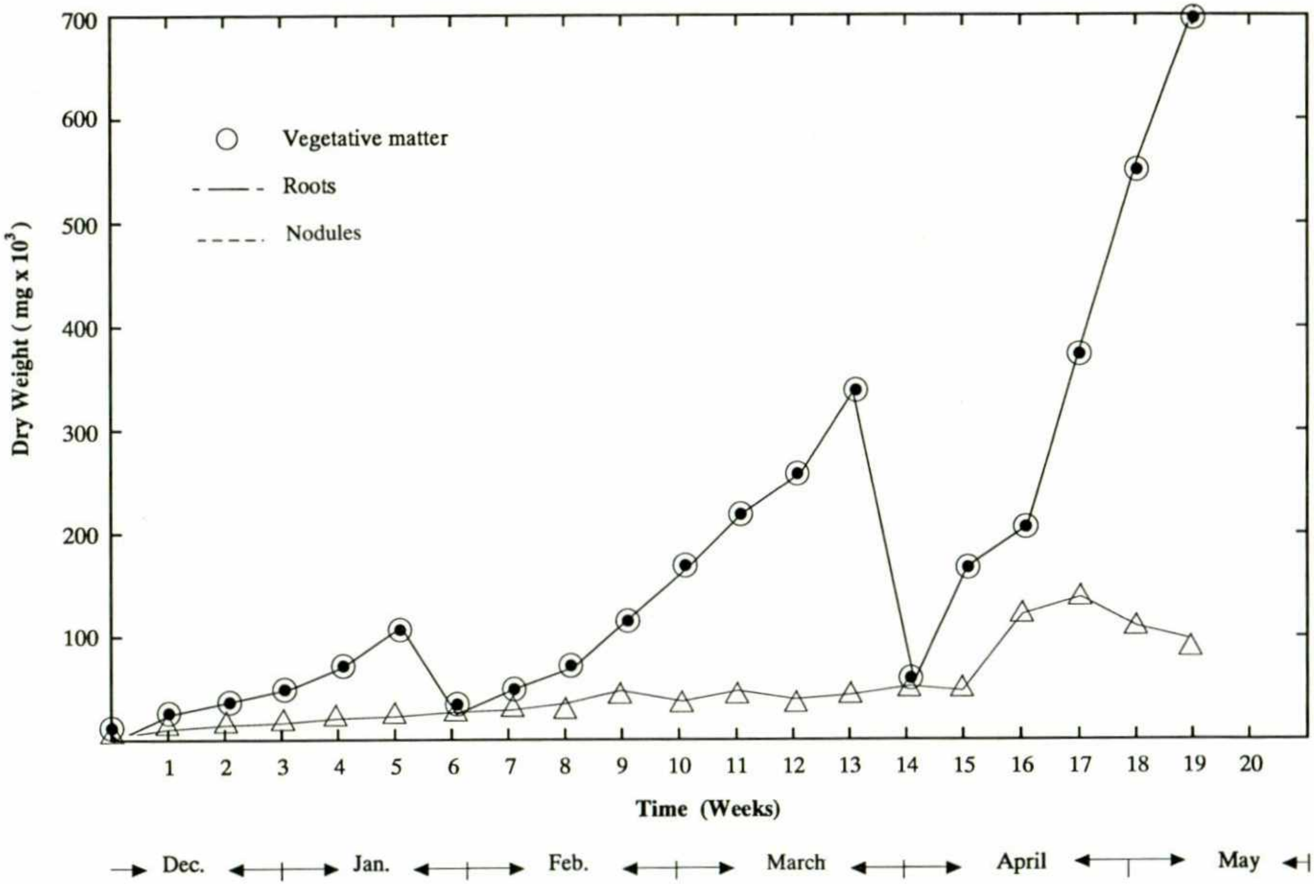

Fig. 1. Seasonal charges in plant components dry weight. Exp. II Arrows indicate harvesting.

periment. The average nitrogen concentration was 2.95, 2.23 and 5.52% for vegetative materials, roots and nodules, respectively. This correspond to a crude protein content of 18.4% in the vegetative materials. Nelson *et al* (6) reported that crude protein content of Egyptian Clover ranged from 8.1 to 18.0%. Nelson *et al* (6) also reported that crude protein content of Egyptian Clover ranged from 8.1 to 18.0%. Moreover, Nelson *et al* (6) reported that early seeding and early stage of harvesting resulted in the highest protein content. In comparison oat hay at the milk stage had 7.8% protein (6). These data combined together suggested that Egyptian clover is a dependable crop in producing good forage yield with a high nutritive value, which makes it a suitable winter forage crop. This is in addition to its ability in increasing soil N content after its roots decomposition.

Estimates of the amount of nitrogen fixed were obtained by subtracting the total nitrogen in the whole plant from the corresponding figure at the following harvesting, and the amount of nitrogen transferred by subtracting the nitrogen content of the root plus shoot from the corresponding figure at the following harvest. Nodule efficiency (milligrams nitrogen fixed per gram dry weight of nodules per day) was calculated by the net assimilation rate formula of Williams (8) for the harvest periods, using the total N content data. However, because the decrease in nodule weight and nitrogen content following harvesting, estimation of N fixation for this period was eliminated.

Nodule efficiency in fixing nitrogen range was 18.31-68.83 mg N/gm dry weight

Table 4 — Symbiotic nitrogen fixation, transfer and nodule efficiency of Egyptian clover.

Period	Nitrogen fixed/ Plant	Nitrogen Transferred Nodules to of N fixed	Nitrogen Transferred red as % plant.	Nitrogen wt. of Nodules/	Mean dry nodule/ day.
	mg		%		mg
1-2	2.51	2.18	86.9	10.64	33.07
2-3	3.08	2.92	94.8	15.25	28.85
3-4	7.00	6.52	93.1	21.30	46.95
4-5	12.66	12.14	95.9	30.49	59.32
5-6	7.53	7.05	93.6	40.13	26.81
6-7				40.29	
7-8	8.95	8.16	91.2	40.79	31.35
8-9	8.93	8.92	99.9	57.79	22.08
9-10	13.60	13.04	95.9	67.54	28.77
10-11	9.76	9.64	98.8	64.66	21.56
11-12	8.53	8.27	97.0	66.57	18.31
12-13	20.07	19.51	97.2	70.21	40.84
13-14	20.13	19.59	97.3	76.21	37.73
14-15				62.22	
15-16	20.91	20.79	99.4	55.24	54.08
16-17	30.38	29.92	98.5	63.05	68.83
17-18	35.24	34.76	98.6	75.33	66.83
18-19	33.23	33.02	99.4	87.33	54.36
19-20	32.87	32.00	97.4	101.77	46.14
mean	16.20	15.79	96.2	55.10	40.35

nodule/ day with an average of 40.35 mg N/gm dry wt. nodule/ day (Table 4). Wilson (9) using the same method in estimating nitrogen fixation capacity of some legumes reported that the range of nodule efficiency of early inoculated plant of *Glycine wightii* Cv. Cooper and *Phaseolus atropupureus* Cv. Siratro was 54-56 and 48-59 mg N/gm dry weight nodule/ day, respectively. Moreover, Table 4 showed that never less than 87% and frequently almost all the nitrogen fixed symbiotically was transferred from the nodules to the other plant parts soon after fixation.

These results were similar to those of Pate (7) who found that 90% of the nitrogen fixed in nodules of pea and vetches was transferred to the tops. These data collectively suggest that nodule development and the potential for nitrogen fixation in this crop is sufficient to sustain high yield. Finally, it is suggested that the effect of seeding date on forage yield and the trend of water soluble carbohydrate reserve of this crop should be further investigated.

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نمو وتكوين العقد البكتيرية في البرسيم المصري وقدرته على تثبيت النيتروجين

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أجريت تجارب لغرض تقييم محصول البرسيم المصري (صنف مسقاوي) كمحصول علف شتوي تحت فترتي حصاد (3 ,6 أسابيع) وقدرته على تثبيت النيتروجين تكافلياً. لقد أوضحت فترتا الحصاد فروقات معنوية في كل من إنتاج العلف خلال كل فترة نمو وإنتاج العلف خلال موسم النمو وإنتاج البذور. وتشير هذه النتائج إلى عدم الحصول على الإنتاج الأقصى من هذا المحصول ما لم يتم استعمال فترة حصاد مناسبة. لقد أعطى هذا المحصول إنتاجاً أعلى من العلف والبذور عند حصاده كل 6 أسابيع مقارنة بحصاده كل 3 أسابيع.

لقد قدّر التثبيت النيتروجيني التكافلي لهذا المحصول بطريقة المحتوى النيتروجيني الكلي. لقد تكونت العقد البكتيرية مبكراً في هذا المحصول. وأما متوسط البروتين في المجموع الخضري لهذا المحصول فقد كان مساوياً 18.4%. إلى جانب هذا، فقد كانت كفاءة هذا المحصول في تثبيت النيتروجين تكافلياً هي 40.35 مجم ن/ جم عقد بكتيرية جافة/ يوم. لقد استخلص من هذه النتائج أن البرسيم المصري محصول علفي شتوي يمكن الاعتماد عليه في الحصول على إنتاج علفي جيد وذي قيمة غذائية عالية تحت إدارة جيدة. كما أن قدرة هذا المحصول على تثبيت النيتروجين تكافلياً تكون كافية للمحافظة على إنتاج مرتفع تحت الإدارة الجيدة لهذا المحصول. وأخيراً، فلقد اقترح أنه يجب أن يدرس تأثير مواعيد الزراعة على إنتاج هذا المحصول وكذلك تقييم ميل المواد السكرية التي تذوب في الماء لهذا المحصول.