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EFFECTS OF WATER STRESS, TEMPERATURE, PROLONGED DARKNESS AND PODS ON PHOTOSYNTHESIS AND RESPIRATION OF INDIVIDUAL LEAVES OF *Vicia faba*[1].

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1. INTRODUCTION

Carbon balance plays an essential part in the prediction of dry matter production. Therefore, information on how plants change their ability to capture and to release CO_2 in response to physiological and environmental factors is vital for the development of simulation models. Although a large amount of studies have been done on the two major components of plant carbon balance, photosynthesis and respiration of plants growing under different environmental conditions, few studies have considered that leaves developed in different phenological phases may have different responses to changes in the environment (*1, 10*). Both photosynthesis and respiration of higher plants have been linked to factors such as environmental conditions, nitrogen content, growth rate and energy demand (*4, 9*). More recently, the control of photosynthesis and respiration in higher plants has been based on two main hypotheses: the

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availability of substrate (source-limited) and the energy demand (sink-demand). During the different phenological phases of a plant, both the sources and the sinks can present a great amount of variation. Therefore the ratio photosynthesis: respiration may present changes during the life cycle of a plant due to that variation. Disregarding such changes may lead to a wrong conclusion about the link between photosyntheses, respiration and yield.

The main objective of this study is to assess the effects of changes in environmental factors, sources and sinks on the respiration and photosynthetic rates of *Vicia faba* leaves developed at different phenological phases.

2. MATERIAL AND METHODS

Plants were grown in 26 cm diameter and 23 cm depth plastic pots filled with a 2:2:1 mixture of vermiculite, sand and gravel. Five pre-germinated seeds were planted per pot on April 28, 1992. The average glasshouse temperature was kept around 20° C. The treatments consisted of an irrigated and a dry treatment.

The irrigated plants were irrigated daily early in the morning and late in the afternoon. To avoid the acute water stress which occurs when dry conditions are imposed on plants growing under a controlled environment, the water stress in this experiment was only applied during the life span of different leaves considered. At the time of unfolding leaf 7, during the vegetative phase, 25 pots were labelled and left without any irrigation. Ten measurements of leaf respiration were taken, at two day intervals, in each treatment, from unfolding to full expansion of leaf 7. After full expansion, the dry plants were rewatered and measurements followed for one more week at two day intervals. Measurements of leaf area and leaf dry weight were taken at the same time. The same procedure was followed for leaf 15 (developed at the flowering phase).

To test the effects of sinks (pods) on leaf respiration rate, pods were removed in a set of plants (6 pots) from both irrigated and dry treatments. The measurements were taken from unfolding to maturity of leaf 26 (developed at the podfilling phase) as described above. At that time (86 days after planting (DAP)), the plants had an average of 16 pods which represented around 12% of the total plant dry weight.

The photosynthesis light response curve was taken at the time of full expansion of leaves 7, 15 and 26 (with and without pods). An average of five measurements, using an open gas exchange system (LCA; ADC Ltd.) fitted with a light unit (12v, 100W) were taken in each treatment.

At the time of full expansion of leaves 7, 15 and 26, five pots of each treatment were placed in a dark room, set with the same average temperature as that in the glasshouse, and the leaf respiration rate was measured at 12 hour intervals.

The response of leaf respiration to increasing temperature was investigated, at the time of full expansion of leaves 7, 15 and 26, by setting the glasshouse temperature, in the morning (around 8:00 a. m.), at its minimum and increasing the temperature by around 10° C each two hours. The respiration measurements were taken after the plants were left for two hours in each temperature. The range of temperature obtained was between 5 and 40° C.

Statistical analyses were conducted using the Statistical Analyses System (SAS Institute Inc, Cary, NC, USA). The graphs are presented with a mean and a standard deviation of the mean.

3. RESULTS AND DISCUSSION

3.1. *Effects of Water Stress and Rewatering*

The variation in respiration rate and area of leaves developed in the vegetative phase (leaf 7) and flowering phase (leaf 15) are shown in Figures 1 a-b. Despite a tendency for an increase in area of leaf 7 after rewatering, changes in respiration were not so clear (Fig.1a). In contrast, a leaf developed during the flowering phase (leaf 15) showed a notable increase in both respiration and area after rewatering (Fig.1b).

The increase in both leaf respiration rate and leaf area just after irrigation of a water stressed leaf developed during the flowering phase (leaf 15), suggests that under water stress the plant is able to store photosynthate and to use it for the growth process on rewatering. Similar results have been found in other studies (*5*, *6*). The fact that leaf 7 did not show any response on rewatering indicates that more studies are clearly needed in this particular area. However, two speculative points can be discussed as possible reasons for this behaviour. Firstly, the low amount of radiation available at the time of growth of leaf 7. The daily average radiation was 3.2 MJ m^{-2} during the vegetative phase and 8.0 MJ m^{-2} during the flowering phase. Secondly the higher growth activity in the vegetative phase compared with the flowering phase (*3*). Clearly, both factors would contribute to a low amount of carbohydrate being stored during the vegetative phase, a fact which may be the explanation for the lack of response of leaf 7 on rewatering.

3.2. *Effects of Pods*

Respiration rate of a leaf developed during the podfilling phase (leaf 26) did not show any significant differences ($p<0.05$), for both irrigated and dry Treatments, between the plants with and without pods (Fig.2a-b).

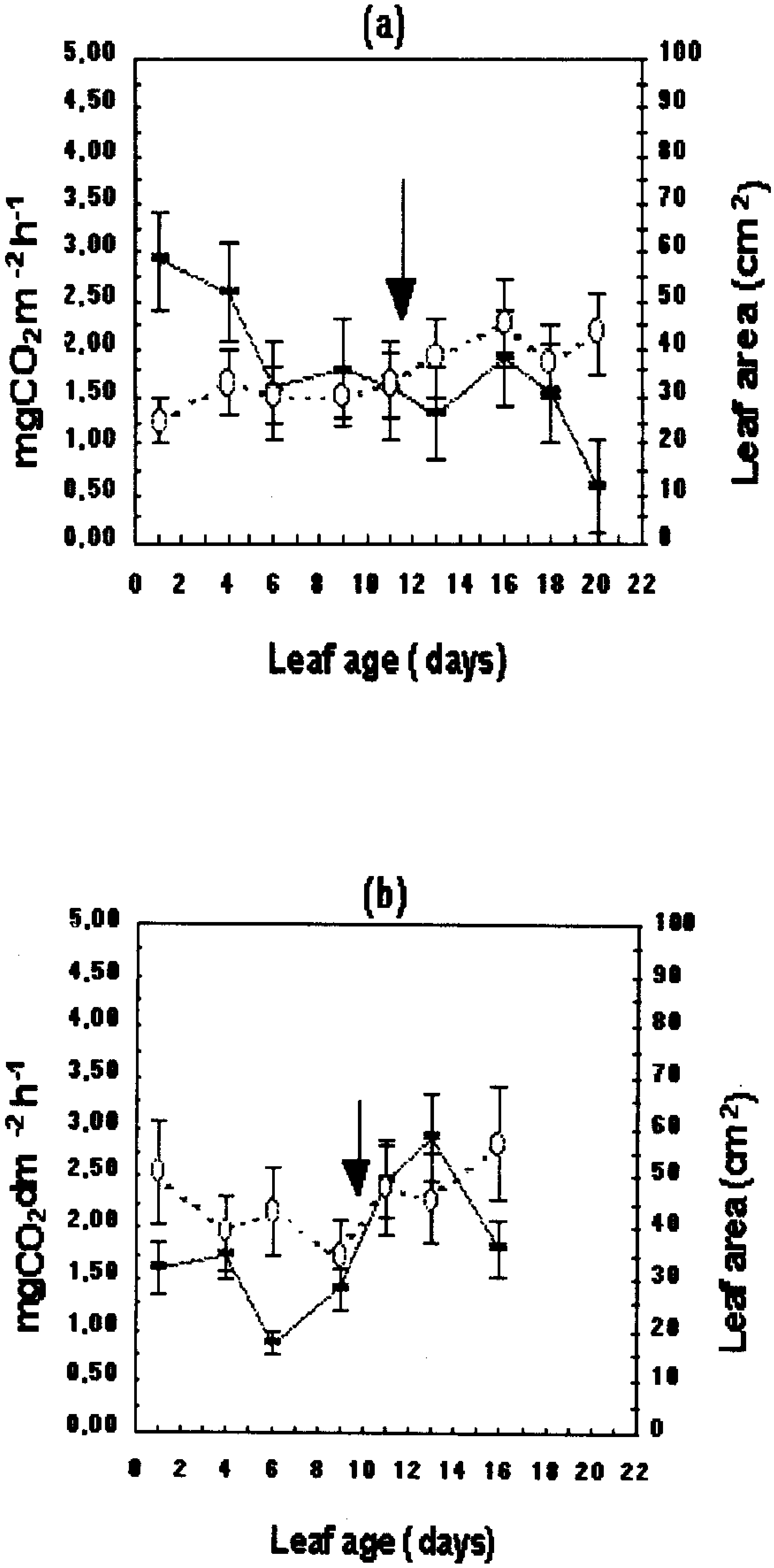

FIGURE 1 - Rate of CO_2 released per unit of area (-) and leaf area expansion (0) of leaf 7 (Fig. 1a) and leaf 15 (Fig. 1b). The arrow indicates the time of rewatering.

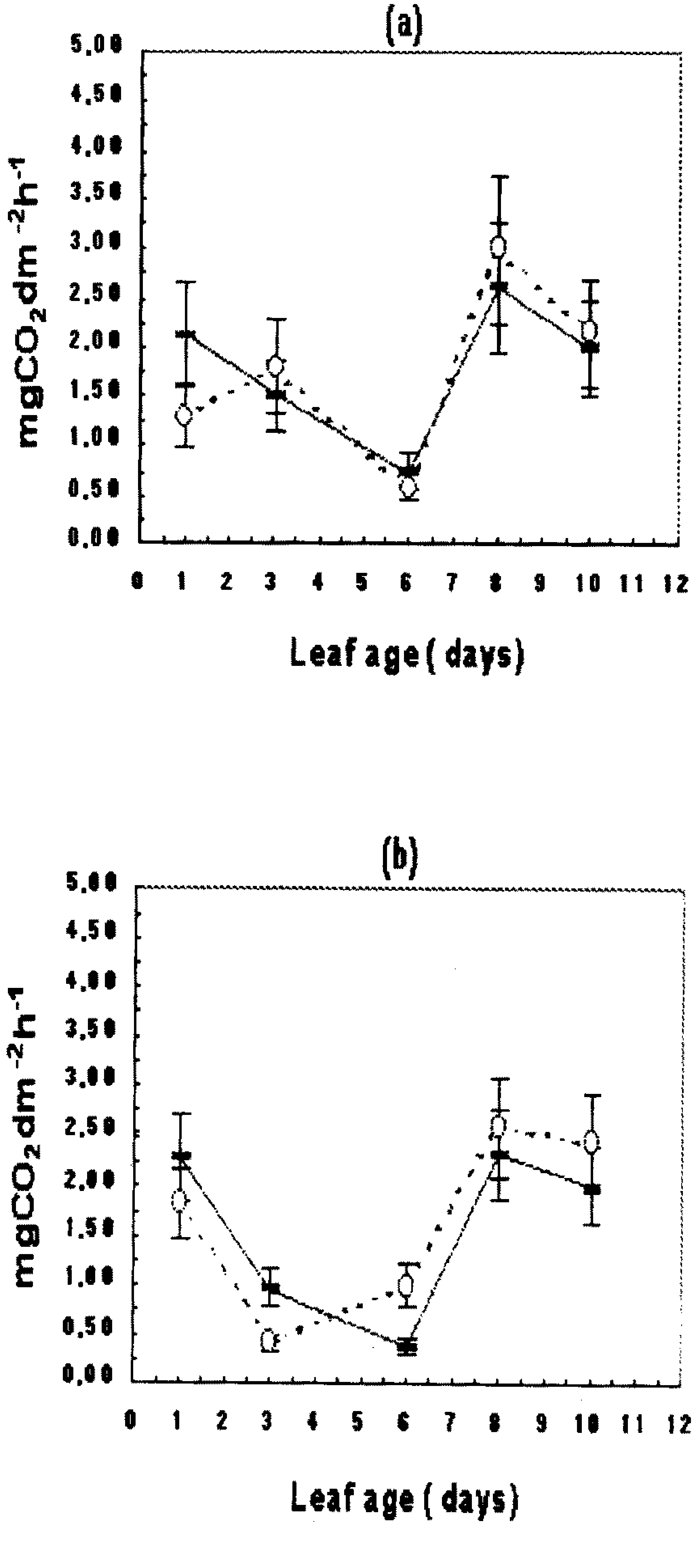

FIGURE 2 - Rate of CO_2 released per unit of area of leaf 26, with (-) and without (0) pods for the irrigated (Fig.2a) and dry treatments (Fig.2b).

3.3. *Effects of Temperature and Pods*

The effects of temperature upon respiration rates of individual leaves (leaves 7 and 15) are shown in Figures 3.a-b and Table 1. There was an increase of respiration with increasing temperature. No significant differences ($p<0.05$) were found between the irrigated and dry treatments for a leaf developed at both vegetative (leaf 7) and flowering phase (leaf 15).

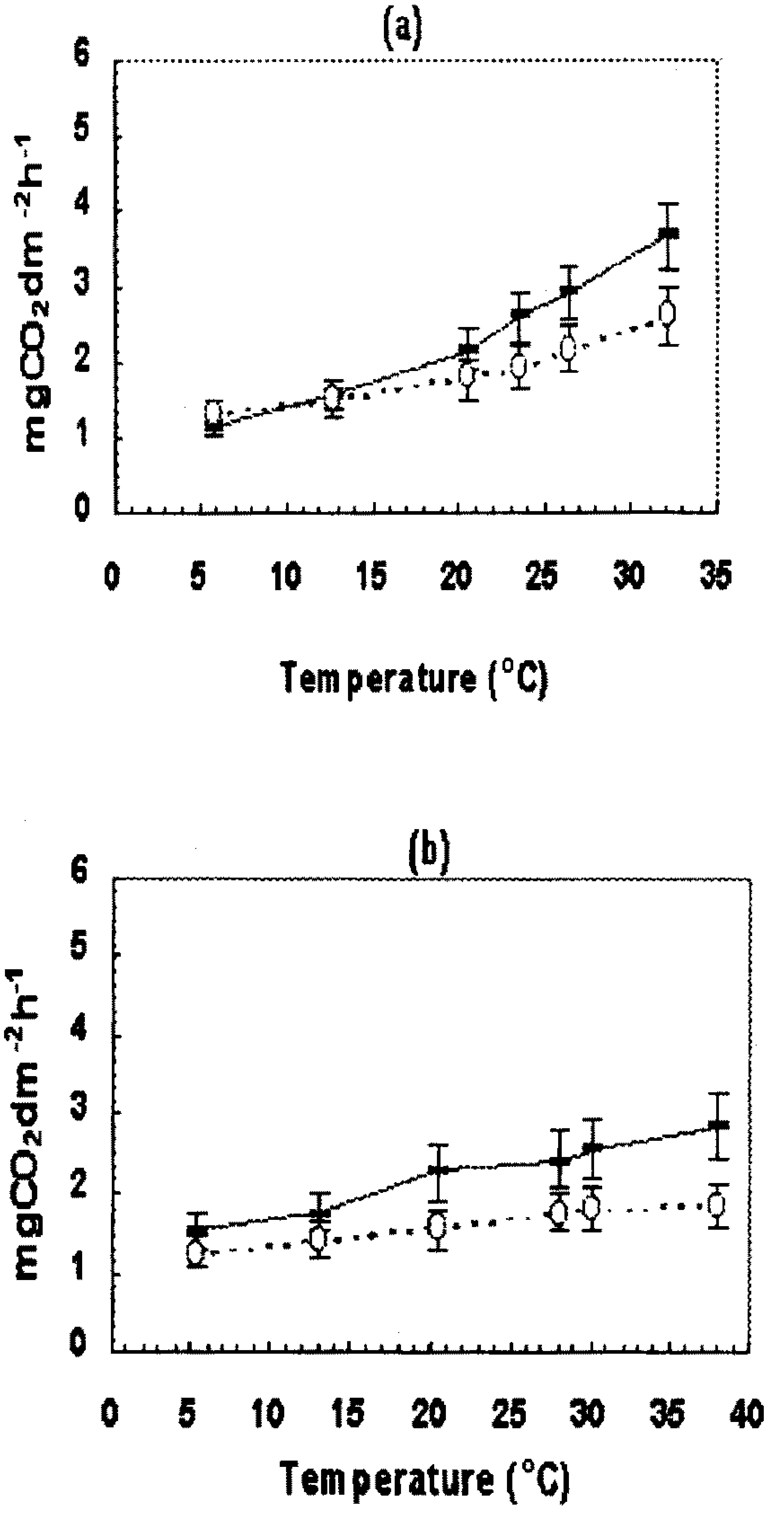

FIGURE 3 - Response of leaf respiration to increasing temperature for the irrigated (-) and dry (0) treatments for leaf 7 (Fig.3a) and leaf 15 (Fig.3b).

The results for leaf 26 show that while the irrigated plants without pods responded to increasing temperature with a strong increase in respiration rate, the irrigated plants with pods showed a smaller response. However, they also showed a strong increase between 25 and 30 °C (Fig.4.a-b). Considering the whole temperature interval, the leaves from an irrigated plant without pods had a Q_{10}, the factor by which the respiration increases for a temperature increment of 10 °C, of 2.6 whereas the irrigated plant with pods had a Q_{10} of 1.4. There were no significant differences ($p<0.05$) due to temperature between the response of a dry plant with and without pods. It can also be seen from the results that both irrigated and dry plants with pods showed a greater rate of respiration at low temperature than the plants without pods. The response of leaf 26 from the irrigated treatment to increasing temperature is significantly greater ($p<0.05$) than from the dry one (Fig.4.a-b; Tab.1).

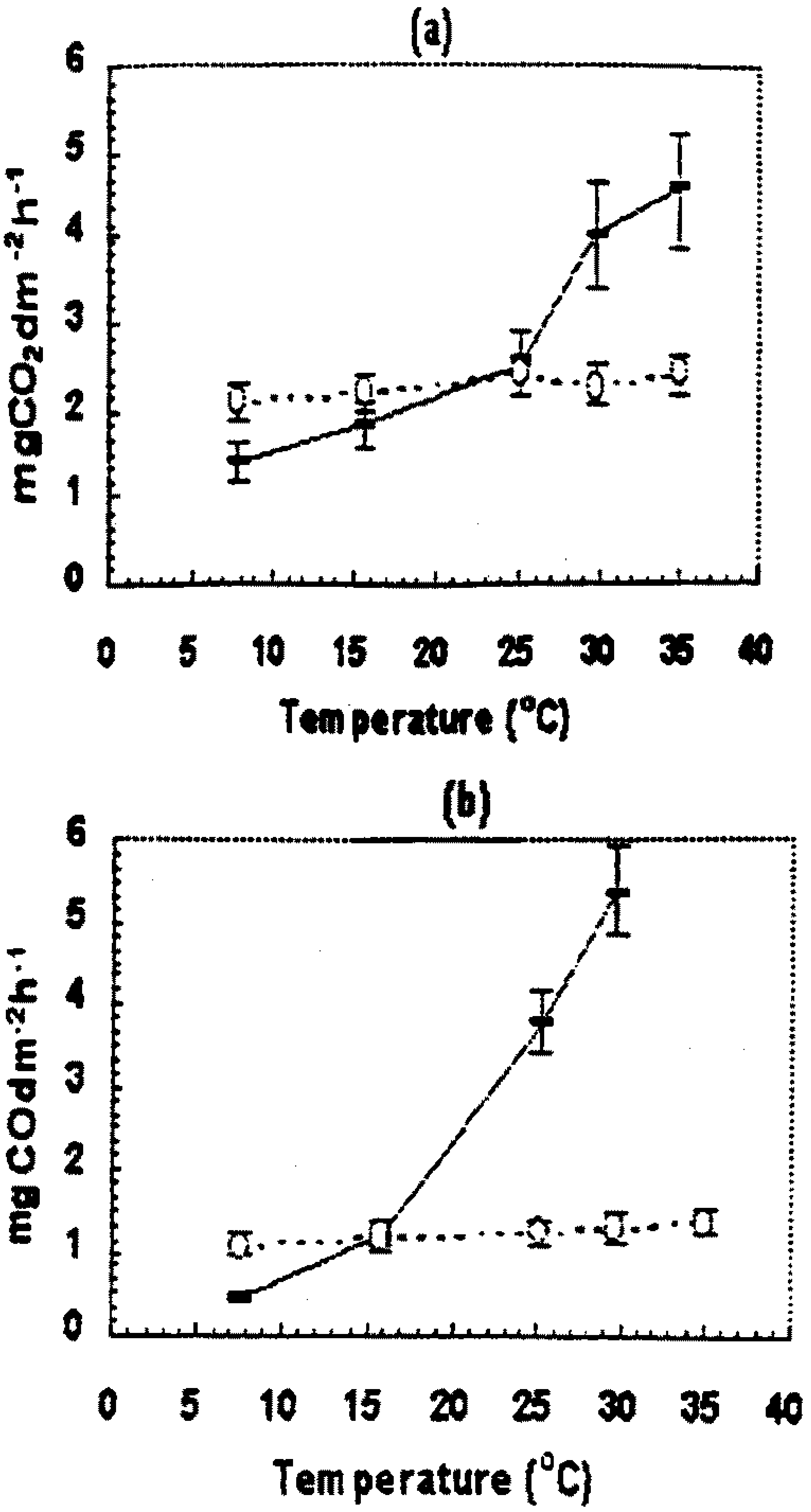

FIGURE 4 - Response of leaf respiration to increasing temperature for the irrigated (-) and dry (0) treatments for leaf 26 with (Fig.4a) and without pods (Fig.4b).

TABLE 1 - Values of Q_{10} of individual leaves of *Vicia faba* for the irrigated and dry plants with pods, derived on the range 5 to 40° C.

Leaf Number	Irrigated	Dry
7	1.50 ± 0.30	1.30 ± 0.11
15	1.20 ± 0.20	1.20 ± 0.10
26	1.40 ± 0.11	1.10 ± 0.16

Leaves developed during the vegetative and flowering phases showed a response to temperature in the lower ranges of the values reported in other studies (*1*, *7*, *11*). This leads to two conclusions. Firstly, they may confirm the fact that respiration response to temperature decreases with increasing growth temperature. Secondly, they are in line with, but do not prove, the theory which suggests that the effect of substrate on respiration rate is stronger when its level is limiting. As the leaves were growing in a low light environment, the level of substrate available for respiration is expected to be low. Therefore they could not respond to increasing temperature by increasing their rate of respiration by the same degree as has been reported in other studies. The higher response of leaf respiration rate from depodded plants under irrigation further supported this theory. It is reasonable to assume that leaves from plants without pods will accumulate a higher amount of substrate and use it in a situation of higher respiration. Furthermore, the daily average of radiation during the development of leaf 26 was 14 MJ m^{-2}.

3.4. *Effect of Prolonged Darkness*

The time course of CO_2 released from the different leaves under irrigated and dry treatments is presented in Figures 5.a-b. The respiration rate of leaves from both irrigated and dry treatments showed a steady decrease from zero to 48 hours in darkness, when they stabilized. Despite similarity between treatments, some particular points can be noticed. The respiration rate of the dry treatment leaves showed a greater decrease in the first 12-24 hours than the irrigated one. With 36 hours in darkness the dry treatment leaves had almost reached their "maintenance level", whereas the irrigated leaves reached their lowest level after 48-60 hours in darkness. The results show that there were no significant differences ($p<0.05$) neither between leaves in the same treatment nor between different treatments. However, the sensitivity of the infra-red gas analysis system to detect small differences in CO_2 concentrations could have had an effect on these results. When the results are presented per unit of dry mass at

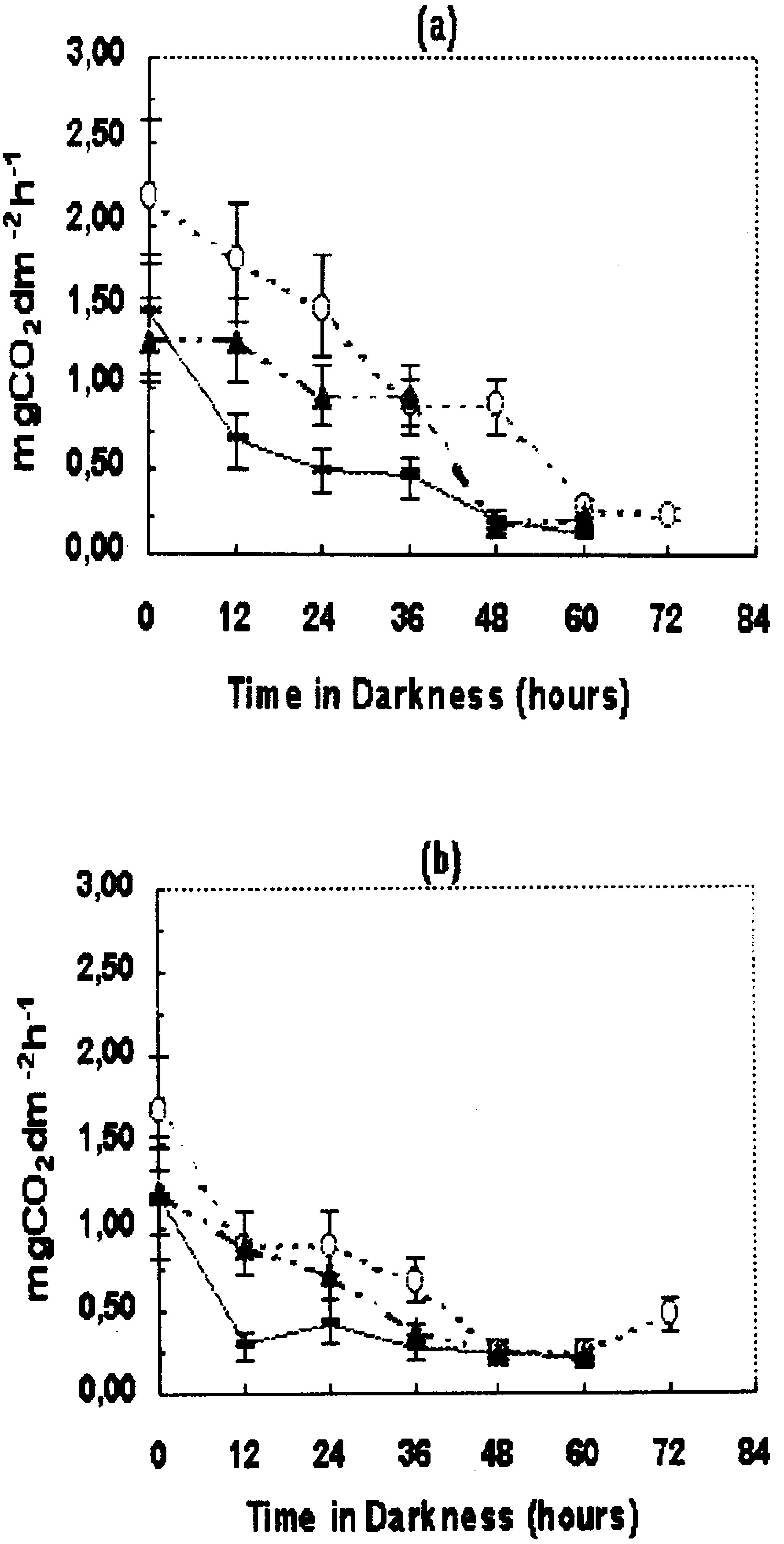

FIGURE 5 - Response of leaf respiration rate to prolonged darkness for the irrigated (Fig. 5 a) and dry (Fig. 5 b.) treatments. (-) leaf 7; (0) leaf 15 and (▲) leaf 26.

the end of the dark period (Table 2), no significant differences ($p<0.05$) are shown between leaves developed at different phenological phases or between treatments.

The effects of prolonged darkness on individual leaves from both irrigated and dry treatments showed that the respiration levels reached by the leoves after 60 hours in darkness were not affected by the rate of leaf respiration before darkness. However, the initial level of leaf respiration affected the decay in the first 24 hours. Similar results of the decline of leaf respiration under prolonged darkness and the values of maintenance respiration found in this study have also been found by theoretical calculation and experimental procedure in *Vicia faba* leaves (*2*).

TABLE 2 - Maintenance respiration, per unit of dry mass, of individual leaves of *Vicia faba* for the irrigated and dry treatments

Leaf Number	Irrigated $mgCO_2(g\ dwt)^{-1}day^{-1}$	Dry $mgCO_2(g\ dwt)^{-1}day^{-1}$
7	14.0 ± 3.1	14.0 ± 4.2
15	18.0 ± 4.1	17.0 ± 3.4
26	16.0 ± 2.9	13.0 ± 2.6

3.5. *Photosynthetic Response*

Water stress decreased the leaf photosynthetic capacity of a young, fully expanded leaf developed at vegetative (leaf 7), flowering (leaf 15) and podfilling phases (leaf 26). The maximum photosynthetic capacity (A_{max}) values were significantly lower, by 2 to 4-fold, for the dry leaves. The decrease in initial light use efficiency (ε) was only significant in leaf 15 and 26 (Tab.3). The presence of pods had a strong effect on A_{max} and ε in the irrigated plant. However, the results were only significant for A_{max} (Tab.3). The dry plants did not show any significant response in leaf photosynthetic capacity due to the presence of pods (Tab.3).

The results showing that leaf respiration rate was not affected by the presence of pods, whereas the leaf photosynthetic capacity was, again lead to two main conclusions. Firstly, these results might be light dependent, i.e., under a high light level and hence high assimilation, the results may show on respiration. Secondly, it may be that most of the energy needed for pod growth is generated in the pod itself, and therefore leaf respiration will be unaffected by the presence of pods.

Some other studies have shown different effects of sink and growth environment on respiration rate. For example, instantaneous respiration rate of decapitated sunflower was around 4 times lower than for the intact plant, for both irrigated and dry treatments (*12*). Also some results have showed that the combination of different levels of temperature and radiation had a significant effect on photosynthesis, respiration and relative growth of potato (*8*).

Respiration rate measured over 16 hours at 30 °C was greater in plants which have been previously exposed to low temperature and high light than for plants exposed to high temperature and low light. Net photosynthetic rate was greater for plants from hot rather than cool environment. The highest relative growth rate was obtained for plants growing at low temperature and light level.

The results presented and discussed here further stress the complexity of the process involved in the control of photosynthesis and respiration of higher plants. They also imply that the empirical approach used in crop modelling, dictating that a given amount of photosynthesis results in a given amount of respiration, does not hold under all conditions.

TABLE 3 - The rate of net photosynthesis at saturated light intensity, A_{max} (μmol CO_2 m^{-2} s^{-1}) and the initial light use efficiency, ε (μmol PAR m^{-2} s^{-1})$^{-1}$) obtained from the fitted light response curve of individual leaves of *Vicia faba*. For a leaf developed at the podfilling phase, the treatments are irrigated and dry with pods (irr.(pods); dry(pods)), and irrigated and dry without pods (irr. and dry). The 95% confidence interval (C.I.) is also given.

Treatments	A_{max}	95% C.I.	ε	95% C.I.
		Leaf 7		
Irr.	21.8	20.2	0.03	0.020
		23.5		0.040
Dry	10.9	8.5	0.02	0.009
		13.3		0.030
		Leaf 15		
Irr.	19.4	17.9	0.03	0.020
		20.9		0.040
Dry	6.0	4.8	0.01	0.004
		7.2		0.010
		Leaf 26		
Irr.(pods)	24.3	20.8	0.03	0.020
		27.8		0.040
Irr.	17.4	14.6	0.02	0.005
		19.1		0.030
Dry(pods)	5.6	5.0	0.01	0.006
		6.2		0.020
Dry	4.7	4.2	0.01	0.006
		5.3		0.020

4. SUMMARY

Respiration rate and photosynthesis of individual leaves developed at different phenological phases were followed in irrigated and water stressed plants of *Vicia faba* L., growing under controlled conditions. The results showed that water stress decreased the photosynthetic capacity of all leaves considered. However, it had no effect on the respiration rates of the same leaves. Also the presence of pods had an effect on the photosynthetic rate of a leaf developed during the podfilling phase, but did not affect its

respiration rate. Respiration rate of irrigated and water stressed leaves developed at the vegetative and flowering phase did not show any significant difference ($p<0.05$) with increasing temperature. However, a difference was shown for a leaf developed at podfilling phase. The maintenance respiration was shown to be statistically ($p<0.05$) the same for all leaves in both treatments.

5. RESUMO

(EFEITO DO ESTRESSE HÍDRICO, TEMPERATURA E AUSÊNCIA DE LUZ NA FOTOSSINTESE E RESPIRAÇÃO DE FOLHAS INDIVIDUAIS DE *Vicia faba*)

Medidas da taxa de respiração e da capacidade fotossintética de folhas individuais de *Vicia faba* L., crescendo em ambiente controlado, foram obtidas durante diferentes fases fenológicas da cultura. Os resultados mostraram que o estresse hídrico reduziu a taxa fotossintética, mas teve pequeno efeito na taxa de respiração das folhas consideradas. Da mesma forma, a presença de vagens afetou a taxa fotossintética da folha desenvolvida durante o enchimento de grãos, mas não afetou a sua taxa de respiração. Folhas de plantas irrigadas e sob estresse hídrico, desenvolvidas durante a fase vegetativa e de florescimento, apresentaram a mesma resposta com o aumento de temperatura. No entanto, folhas de plantas irrigadas desenvolvidas durante a fase de enchimento de grãos, apresentaram resposta mais acentuada que as não-irrigadas com o aumento de temperatura. A respiração de manutenção foi a mesma, em ambos os tratamentos, para as folhas desenvolvidas em diferentes fases fenológicas.

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