

SCIENTIFIC CONTRIBUTIONS**Effects of chilling stress on leaf photosynthesis of sunflower.**

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To simulate the effects of a possible decreasing of night temperatures in sunflower a pot experiment was carried out in 1991 at Pisa University Agronomy Department. A short period of cold stress was imposed by transferring the plants from 30° C air temperature to 10° C for 24 hours. After this period plants were transferred again in an air regime of 30°C. Gas exchange measurements were performed before and after cold stress on plants at three stages of development.

Sunflower showed a very rapid and marked response to cold stress which was accompanied by changes in photosynthetic capacity. CO₂ assimilation rate and all the other parameters recovered rapidly when the plants were carried in normal air temperatures. The depression of photosynthesis following chilling stress was increasingly higher as development progressed; the plants presented a lower capacity to recover in maximum photosynthetic activity after flowering when the leaves regained only 30% of the full photosynthetic capacity. Transpiration rate returned almost to the same values of the control.

No statistical differences were observed in the main productive characteristics (seed yield, oil content, number of seed per head) and in the protein content of the seeds between stressed and unstressed plants.

KEY WORDS: sunflower; chilling stress; photosynthesis; seed yield.

INTRODUCTION

All physiological processes vary more or less with temperature. It is therefore important to determine mechanisms responsible for cold sensitivity among the processes which are highly sensitive to temperature and are important for yield formation. Physiological dysfunction occurs in many plants when they are subjected to temperatures of 10-12°C or lower but above freezing. Chilling temperatures produce problems mostly in metabolic systems associated with growth and development. Photosynthesis is strongly inhibited by low temperatures (Levitt, 1972) and little information is available concerning the interaction of temperature with CO₂ and water vapour exchanges in sunflower. Low night temperatures could influence sunflower seed yield by decreasing production of photosynthate or by altering efficiency of photosynthate partitioning within the plant. Goyne *et al.* (1978) found that temperature, mainly after flowering, has a dominant effect on sunflower yield, oil quality and oil quantity.

With the aim to understand how low temperatures (ca. 10°C) - which could occur during sunflower growing season - may limit sunflower productivity, a short period of chilling stress was imposed by transferring the plants from 30° C air temperature in light to 10° C in dark for 24 hours. Gas exchange measurements were performed before and after chilling stress on plants at three stages of development.

MATERIALS AND METHODS

Trials were carried out in 1991 at Pisa University Agronomy Department. Seeds of sunflower (*Helianthus annuus* L.) cv. Gloriasol were sown on 6 May in 35 cm diameter pots with a capacity of over 5 liter and filled with sand and compost mixture soil (1:2). Plants were grown outdoors under full sunlight; a complete fertilizer was applied soon after emergence and plants were watered daily.

Phenological observation were made according to Cetiom, 1983.

At each stage of development a short period of cold stress was imposed by transferring the plants from 30°-35°C air temperature in light to 10° C in dark condition in a controlled growth chamber with relative humidity of 80%.

Gas exchange measurements were carried out before and after stress on plants on July 1 (corresponding to stage E4), July 25 (corresponding to flowering stage *i.e.* F1-F2) and August 9 (corresponding to maturity *i.e.* Mo). Measurement of CO₂ and water vapour exchange were made on cloud-free days on 8 plants for each stage of development. Leaves were always studied on their natural orientation and light environment and therefore measurements on the two full expanded upper leaves were always taken when photon flux densities were at saturating levels for photosynthesis. Leaves of similar age and exposure were sampled at the same time of day in order to avoid sampling errors.

Leaf gas exchanges were measured using an open differential system (A.D.C. Company, Hoddesdon, U.K.). The system consisted of a small battery-operated I.R.G.A., a leaf cuvette, an air supply unit and a data logger. The cuvette was provided with sensors indicating chamber humidity, temperature and PPFD (photosynthetic photon flux density). An internal fan minimized leaf boundary layer resistance. Air supply unit (A.S.U.) provided a measured flow of air to the system which could be conditioned by removing water vapour, CO₂ or both.

Total seeds weight (g p⁻¹) and oil content (by NMR method) were measured on 20 plants for each stage of development for both the control and the stress. The protein content of the seeds was determined by Kjeldahl (total nitrogen x 6.25).

RESULTS

The characteristic symptom of chilling injury in sunflower was a rapid loss of the leaf turgor and successive wilting when plants were transferred from chamber temperature (10°C) to air temperature (30°C-35°C). Leaf gas exchange measurements, taken immediately after exposure 24-h chilling treatment, showed a very rapid and marked response to cold stress which was accompanied by changes in photosynthetic capacity (fig. 1). CO₂ assimilation rate and the other parameters recovered rapidly when the plants were carried in normal air temperatures (fig. 2 and tabs.1, 2,3). Measurements repeated after one hour demonstrated that gas exchange values did not change respect to those observed immediately after rewarming.

The depression of photosynthesis following chilling stress was increasingly higher as development progressed and the plants presented a lower capacity to recover in maximum photosynthetic activity after flowering when the leaves regained only 30% of their full photosynthetic capacity.

Transpiration rate seems to be less sensitive to chilling stress and at flower bud the recovery was almost on the same values of the control (94%); chilling during flowering and maturity resulted in temporary reduction in leaf transpiration rate which, after rewarming, returned respectively to 80% and 60% of the control levels (tab.2 and fig. 2).

The water use efficiency, similarly to photosynthesis, showed a lower sensitivity to chilling stress before flowering (tab.3).

Chilling stress resulted in a temporary reduction in stomatal conductance (fig.3) and after rewarming an increasing trend was observed followed by a progressive decreasing until a lower plateau level was reached. The values were

50%, 90% and 30% of the control at flower bud, flowering and maturity respectively.

No statistical differences were observed in the main productive characteristics (seed yield, oil content, number of seed per head) and in the protein content of the seeds between control and stressed plants.

DISCUSSION

Chilling injury which can occur at temperatures below 10°C in chilling-sensitive species is frequently associated with loss of turgor. The cause of stomatal opening in chilling sensitive species has not been elucidated so far, but it is most likely connected with changes in the permeability of guard cells (Nobel, 1974) and with the cold inactivation of the K⁺-ATPase pump (Garber, 1977). Stomatal opening due to chilling has been demonstrated for bean leaves (Wilson, 1976), which suggests that the phenomenon is widely recurring in chilling sensitive plants. Data in literature show additional factors of chilling injury such as sensitizing stomata to CO₂ (Drake and Raschke, 1974), decline in the rate of the dark reactions of photosynthesis (Taylor *et al.*, 1974), membrane alterations of chloroplasts (Taylor and Craig, 1971) and photoinhibition by light absorbed in excess of that which can be utilized for normal photochemical reactions (Björkman and Holmgren, 1963). As shown in our analyses stomatal behaviour of sunflower leaves under chilling seems to demonstrate, according to Wilson (1976) and Drake and Raschke (1974), a stomatal opening with loss of turgor. The results seem to suggest that stomatal sensitivity changed under the conditions generated by a short period of chilling stress.

The photosynthetic depression in sunflower after brief exposure to chilling temperatures do not determine any dominant effects on yield and oil quantity. This fact confirms the inconsistent relationships between leaf photosynthetic rate and crop yields. We can conclude that the response of the whole plant to low temperatures is probably a result of the complex behaviour at different levels of plant organization and of imbalances among different physiological processes (Gold and Raper, 1983).

Tab.1 - Leaf net photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in controlled and stressed plants at the different stages of development.

<i>Net Photosynthesis</i>			
<i>Stage of plant development</i>	<i>mean</i>	<i>s.d.</i>	<i>t</i>
E4			
control	16,28	0,156	67,6**
stressed	11,48	0,102	
F1-F2			
control	16,65	0,269	55,0**
stressed	5,88	0,371	
M0			
control	15,43	0,361	63,0**
stressed	3,29	0,279	

** indicate significance at the 0.01 level of probability according to F-test.

Tab.2 - Leaf transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) in controlled and stressed plants at the different stages of development.

<i>Transpiration rate</i>			
<i>Stage of plant development</i>	<i>mean</i>	<i>s.d.</i>	<i>t</i>
E4			
control	11,17	0,084	4,5**
stressed	10,51	0,321	
F1-F2			
control	8,20	0,157	9,8**
stressed	7,20	0,202	
M0			
control	8,90	0,059	62,7 **
stressed	5,54	0,114	

** indicate significance at the 0.01 level of probability according to F-test.

Tab.3 - Water use efficiency ($\text{mmol CO}_2 / \text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$) in controlled and stressed plants at the different stages of development.

<i>Water use efficiency</i>			
<i>Stage of plant development</i>	<i>mean</i>	<i>s.d.</i>	<i>t</i>
E4			
control	1,46	0,019	23,4**
stressed	1,13	0,027	
F1-F2			
control	2,03	0,066	40,1**
stressed	0,67	0,011	
M0			
control	1,72	0,051	43,0**
stressed	0,61	0,027	

** indicate significance at the 0.01 level of probability according to F-test.

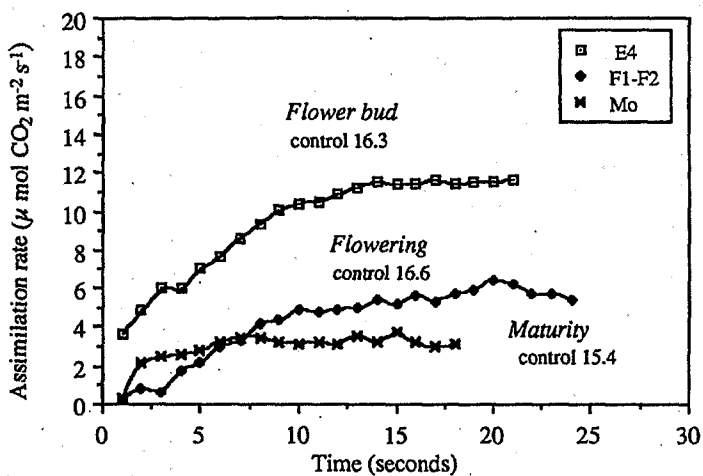


Fig. 1 - CO_2 assimilation rate during recovery at different stages of development. According to *Cetium* E4 flower bud, F1-F2 flowering and Mo maturity.

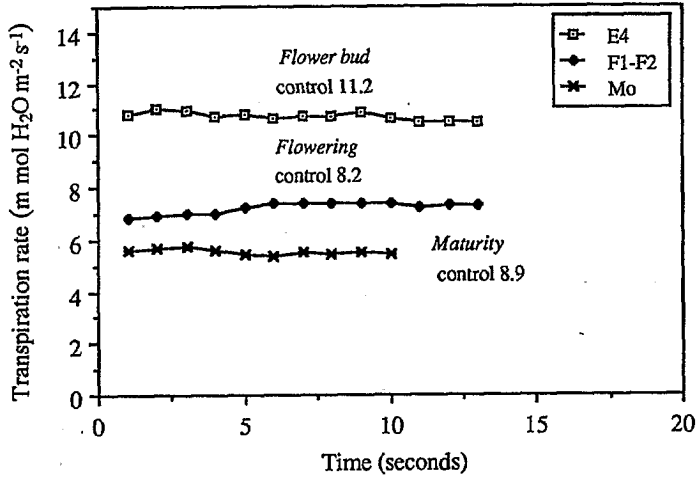


Fig. 2 - Transpiration rate during recovery at different stages of development.

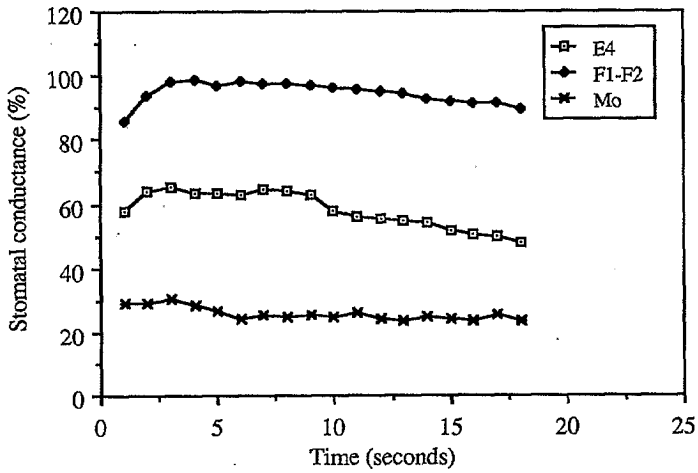


Fig. 3 - Stomatal conductance during recovery at different stages of development. Values are expressed in percentage of the control.

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