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Effect of foliar applications of methanol on growth and yield of sunflower (*Helianthus annuus* L.)

(with 1 figure & 3 tables)

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Abstract. The effect of foliar applied methanol [Met] on growth and development of sunflower (*Helianthus annuus* L.) plants, grown under controlled conditions or in the field, was studied. Foliar sprays of aqueous (30%, v/v) Met were applied at the beginning of capitulum development (23 days from seedling emergence, DFE), and every 4 days up to 41 DFE, when the floret primordia were completely differentiated on the capitulum surface. Met treated plants grown under controlled conditions showed significant changes in vegetative growth and floral development. Met increased stem length by 23.6%, leaf area per plant by 66.5, stem dry weight by 51.4%, number of floret primordia by 46.5% and accelerated completion of floral development by 4.5 days. Met treated plants grown in the field did not show significant changes compared with controls, except subtle differences in vegetative development visually detected in leaf turgor and leaf color 5 days after the treatment commenced, but these changes were not statistically significant.

Key words: Growth, *Helianthus annuus*, methanol, sunflower, yield.
Abreviation: methanol [Met]

Resumen. Se estudió el efecto de la aplicación foliar de metanol [Met] sobre crecimiento y desarrollo de plantas de girasol (*Helianthus annuus* L.) bajo condiciones controladas o en el campo. A partir del comienzo de la formación del capítulo (23 días desde la emergencia de las plántulas, DFE) y cada 4 días hasta 41 DFE, momento en que todos los primordios florales ya se habían diferenciado sobre la superficie del receptáculo, se realizó la aspersión foliar de una solución

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de Met en agua (30%, v/v). Las plantas tratadas bajo condiciones controladas mostraron diferencias morfológicas significativas en crecimiento vegetativo y desarrollo floral. La aplicación de Met aumentó en 23.6% la longitud del tallo, en 66.5% la superficie foliar por planta, en 51.4% el peso seco del tallo, en 46.5% el número total de primordios florales y aceleró en 4.5 días la terminación de la diferenciación floral. En las plantas a campo, la aplicación de Met solamente produjo ligeros cambios en el desarrollo vegetativo, como la turgencia y el color foliar 5 días después de comenzado el tratamiento, pero estas diferencias no fueron estadísticamente significativas.

Palabras clave: crecimiento, girasol, *Helianthus annuus*, metanol, rendimiento.
Abreviatura: metanol (Met)

Foliar applied methanol (Met) has positive effects on growth and development of several plants. In those species having the C_3 photosynthetic pathway such as durum wheat (*Triticum durum*) and barley (*Hordeum vulgare*) (14), mungbean (*Vigna radiata)* (2), tomato (*Lycopersicon esculentum* (15), bachelor's-button (*Centaurea cyanus*) and geranium (*Pelargonium hortorum*) (6) positive growth effects of Met have been described. Nonomura & Benson (14) reported that foliar applied Met increased cotton (*Gossypium hirsutum* L.) dry matter production by 50%, leaf area, turgidity and thickness, hastened maturation and lowered irrigation requirements. They also observed that the treatment increased growth rate and harvest yield, which they attributed, in part, to Met acting as C source (4, 3, 1, 5). Our aim was to determine the effect of foliar application of Met on the early stages of the vegetative and reproductive development of sunflower (*Helianthus annuus* L.).

MATERIALS & METHODS

Experiment 1 (controlled conditions)

Plant cultivation. Sunflower plants, cultivar Dekasol 3881 (Dekalb Seeds of Argentina), were grown in 2.0 L plastic pots filled with good quality garden soil in a controlled environment cabinet with long day (LD) photoperiod (18 + 6 h light/darkness) and 28 ± 2 °C.

Plants were watered daily and fertilized weekly with a N,P,K (20-20-20) compound fertilizer. Minor mineral elements were also added twice a week. During photo-period the quantum flux (400-700 nm) was provided by Sylvania cool white (105W HD) fluorescent tubes and 40W incandescent lamps, with an PPFD at the canopy level of 320 μmol $m^{-1}s^{-2}$.

Met application. Experiments started at floral stage (FS) 2 (Marc & Palmer [11]), 23 days from seedling emergence (DFE), when the apical dome broadens and flattens and the differentiation of

involucral bract primordia is still undetected (11). Four consecutive foliar applications of aqueous (30% v/v) Met without surfactant were made every 4 days ending at 41 DFS (EF 5) when florets on the capitulum surface started to appear at the capitulum rim. Application was made with a hand-held sprayer until run-off and contact of the solution with the soil avoided by covering each pot with plastic film. The time of application in all treatments was 11:00 in the light cycle. Each plant received 15.0 ml of Met during the experimental period. Control plants were treated only with deionized water. Starting 15 DFE, at intervals of 5-8 days and up to 61 DFE, 5 plants per treatment were taken for assessment of the developmental stage of the capitulum. Apex development was followed by periodic sampling, using the ten stages classification of Marc & Palmer (11). The capitula were dissected out and the diameter of each receptacle measured in 3 planes of radial symmetry. Average values and the area of the receptacle were calculated. Floret count on each capitulum and evaluation of capitulum morphology was made at FS 8 (11) using the replica method (8) as modified for sunflower by Hernández & Green (10). A mold of the capitulum surface was made, a resin replica obtained and photographed for floret number count and size measurement.

At harvest [61 DFE] leaf number, stem length and fresh weight of leaves and stems were taken. Leaf and stem dry weight were obtained by oven-drying samples at 60 °C for 48 h. Leaf area was determined with a LI-COR LI-3000A area meter.

Leaf samples from the mid region of upper leaves laminae were taken for anatomical studies, fixed in formalin-acetic-acid-ethanol and embedded in Paraplast. Transverse and paradermal cuts were made on a rotary microtome at 9 µm, stained with safranin-fast green, mounted, and observed with a Nikon Labophot-2 light microscope.

Experiment 2 (field conditions)

Plant cultivation. A field experiment was conducted at the Agronomy Dept., Univ. Nacional del Sur, Bahía Blanca, Argentina (38° 44' Lat. S.; 62° 16' Long. W.). Hybrid cultivar Dekasol 3881 was sown on 18 November on Petrocalcic paleustol plots (four 6 m long rows, spaced 0.60 m apart), arranged in a randomized complete block design with 4 replications per treatment. Water (about 60 mm) was applied at planting for germination and crop establishment. Plants emerged on 23 November and were hand thinned to 5.6 plants.m^{-2}. NH_4NO_3 and P (as triple superphosphate) were applied to the plot area 44 DFE at 122 kg N and 88 kg P.ha^{-1}, respectively. Conventional control measures were taken to minimize insect damage and weed competition. Plots were watered to avoid any significant water deficit.

While growing plants received about 490 mm of irrigation water and 117 mm of rainfall.

Met application. Two treatments (water spray and 30% aqueous Met with no surfactant) were applied to four field replicates, with the same date schedule of Experiment 1, i.e. starting at FS 2 (24 DFE) and ending at FS 5 (42 DFE). At 11:00 the solutions were applied with a hand-held sprayer until dripping from the leaves began. The rate of volume applied per treatment was 200 $L.ha^{-1}$, or approximately 1.2 L per plot. Floral stages were assessed as in Exp. 1. At first anthesis, leaf area and leaf dry weight were measured in 10 plants per each replicate plot. At maturity, 10 plants per replicate plot were harvested from the two central rows to determine stem length, and dry weight, yield components and final grain yield.

Statistics. Statistical analysis followed SAS procedures (16). Data for 5 and 10 replicate plants in experiments 1 & 2 were pooled and subjected to analysis of variance. When F was significant ($P < 0.05$), the least significant difference (L.S.D.) for the comparison of means was determined for each sample (17).

RESULTS

Quantitative changes observed in the vegetative and reproductive growth of plants in both experiments are shown in Tables 1 & 2. In Exp 1 Met significantly increased the receptacle area (Table 1) and the total number of floret primordia without producing significant variations in interprimordial spacing or floret size (Table 1). These responses were observed in all of the Met-treated plants. Under controlled conditions, Met significantly increased stem length, stem dry and fresh weights, leaf area and leaf dry and fresh weights (Table 1). There was also an increase in floral development rate of about 0.10 units of $FS.day^{-1}$ (Fig. 1). The rate increased from 0.25 units of $FS.day^{-1}$ [control] to 0.35 units of $FS.day^{-1}$ [Met treated] (Fig. 1). The final stage of inflorescence formation (FS 10) in treated plants was attained 4.5 days earlier than in the control (Fig. 1). In the field (Exp. 2), yields were generally high in all plots, averaging 362.3 $g.m^{-2}$ [control] and 412.2 $g.m^{-2}$ [Met treated] (Table 2). Foliar applications of Met did not significantly change leaf area and leaf dry weight at anthesis or stem length and stem dry weight at harvest (Table 2). Likewise, the total number of fruits per plant, the number of empty fruits and the 1000 fruit weight showed no significant differences between treated and non treated plants at harvest (Table 2).

Table 1.– Effect of foliar applied methanol on vegetative and reproductive development of sunflower plants grown under controlled conditions (Experiment 1)

Treatment	At Floral Stage 8			At Floral Stage 10					
	Receptacle area (mm^2)	Floret number	Maximum Floret width[b] (μm)	Stem length (cm)	Stem dry weight (g)	Stem fresh weight (g)	Leaf area (cm^2)	Leaf dry weight (g)	Leaf fresh weight (g)
Control	2777.3 (1800)	880 (70)	106.2 (1.1)	21.6 (1.1)	3.7 (0.4)	28.8 (3.2)	737.0 (86)	5.3 (0.4)	37.0 (7.6)
Methanol	9117.0 (1930)	1290 (59)	104.7 (1.3)	26.7 (1.4)	5.6 (0.9)	18.1 (0.8)	1227.0 (260)	7.4 (0.8)	24.8 (1.3)
LSD [a]	4232.4	338	1.1	3.2	1.1	6.8	384.1	1.3	8.2

[a] ($P<0.05$). Standard errors are shown in parentheses. [b] Measured on the first 5 floret primordia of 10 floret rows from the receptacle rim.

Table 2.– Pooled effect of foliar applied methanol on mean yield components of field-grown sunflower plants (Experiment 2).

Treatment	Stem lenght (cm)	Stem dry weight (g)	Leaf area 1st anthesis (dm^2)	Leaf dry weight 1st anthesis (g)	Fruits per plant (No.)	Empty fruits per plant (No.)	1000 fruit weight (g)	Grain yield ($g.m^{-2}$)
Control	147.4 (6.5)	47.7 (5.1)	48.3 (2.8)	31.2 (3.1)	2052 (172)	384 (31)	41.0	362.3 (32.5)
Methanol	148.1 (7.2)	50.8 (3.2)	51.1 (3.3)	38.1 (4.9)	2096 (123)	386 (23)	45.4	412.2 (34.2)
LSD [a]	ns	ns	ns	ns	ns	ns	ns	ns

[a] ($P<0.05$). Standard errors are shown in parentheses.

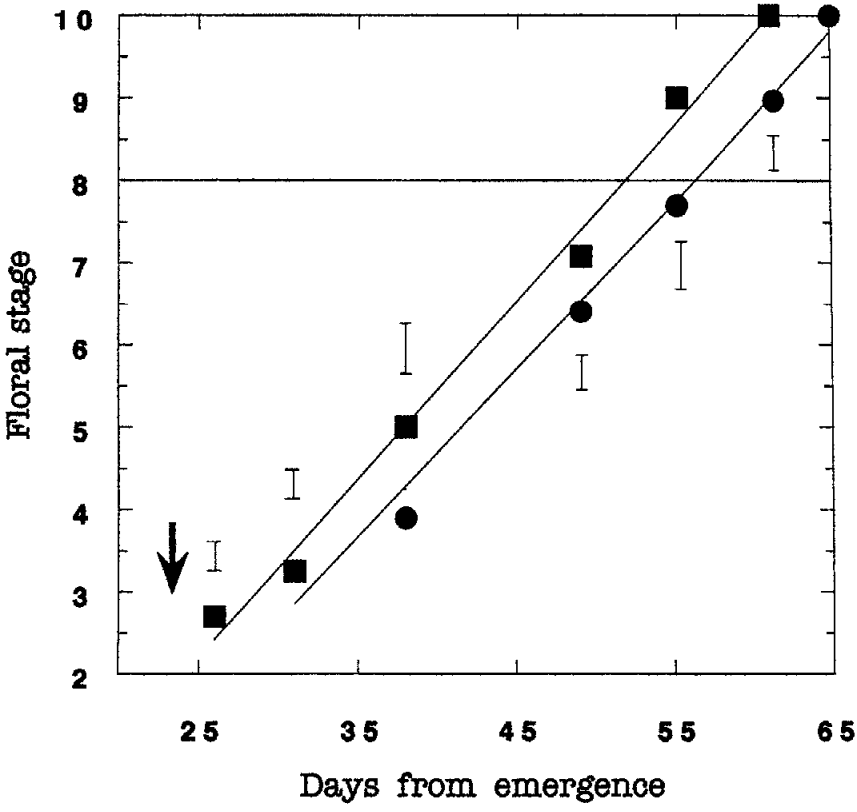

Fig. 1.– Floral stages chronology of sunflower plants growing under LD [18 h] photoperiod. Each point is the mean of 4 plants in two replicates. Vertical bars: L.S.D. Arrow shows beginning of methanol applications. (circles): control plants; (squares): methanol treated plants. Control: FS = -6.84 + 0.45 x DFE (r^2 = 1.00). Methanol: FS = 5.56+0.35xDFE (r^2 = 0.98)

Table 3.– Anatomical changes observed in leaves of control and methanol-treated plants grown under controlled conditions.

Treatment	Upper epidermis thickness (μm)	Palisade layer (μm)	Total leaf thickness (μm)
Control	13.0 (1.6)	74.6 (8.6)	207.3 (20.8)
Methanol	10.6 (1.5)	73.2 (7.9)	206.2 (20.6)
LSD [a]	ns	ns	ns

[a] ($P<0.05$). Standard errors are shown in parentheses.

DISCUSSION

The results show that dry matter accumulation, leaf area and early reproductive development in sunflower grown under controlled conditions responded positively to foliar applications of Met (Table 1) while responses in the field lacked consistency (Table 2). The Met growth stimulation effect, though not of equal magnitude, agrees with that reported by Nonomura & Benson (14). However, in contrast to their results, we did not find any toxic effect of Met when applied to the leaves at a concentration of 30% in any of the experiments. They also reported increased leaf turgidity immediately after foliar application of aqueous 10% to 50% Met solutions on C_3 crops in full sunlight. They proposed that Met could be metabolized to sugar in the leaf with the consequent reduction in leaf osmotic potential, which would lead to increased leaf turgor and subsequently to increased leaf diffusion to H_2O and CO_2. In both our field and greenhouse experiments there was a visible change in leaf turgor following Met application, but leaf water potential measurements made on treated and control plants at different stages of growth (data not shown) were not statiscally different. Nonomura & Benson (14) also reported Met increased cotton leaf thickness by 20 to 50%. Our measurements of leaf thickness showed no differences between treated and non-treated plants (Table 3) and no alterations were noted in the structural integrity of the leaf cuticle. Furthermore, paradermal sections of leaves from plants in Exp. 1 showed a more compact arrangement of palisade layer cells in Met treated plants but no statistical significance was found. Many field studies with foliar applied Met have been reported (1, 12, 18, 13, 7). They also have found no differences in water relations, growth, development, or yield between Met treated and control plants.

Why the plant response was only observed under controlled conditions and not under field conditions is not known. Nevertheless, light quality and ventilation of leaves in the field, reducing the permanence of Met on the leaf surface until some effect on leaf morphology or physiology was produced, could be factors of the differences in response.

The use of Met to increase growth rate may benefit yield of this important C_3 plant when shoot and leaf growth rate become yield limiting factors. Further work is in progress to determine the mechanisms for this response.

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