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The path of carbon in photosynthesis: Improved crop yields with methanol

(food/photorespiration/turgidity/productivity/agronomy)

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ABSTRACT **Foliar sprays of aqueous 10–50% methanol increased growth and development of C_3 crop plants in arid environments. The effects of low levels (<1 ml per plant) of methanol were observed for weeks after the brief time necessary for its rapid metabolism. Within several hours, foliar treatment with methanol resulted in increased turgidity. Plants treated with nutrient-supplemented methanol showed up to 100% increases in yields when maintained under direct sunlight in desert agriculture. In the shade and when winter crops were treated with methanol, plants showed no improvement of growth. When repeatedly treated with nutrient-supplemented methanol, shaded plants showed symptoms of toxicity. Repeated methanol treatments with glycine caused increased turgidity and stimulated plant growth without injury under indirect sunlight, but indoors with artificial illumination, foliar damage developed after 48 hr. Addition of glycerophosphate to glycine/methanol solutions allowed treatment of artificially illuminated plants indoors without injury. Plants with C_4 metabolism showed no increase in productivity by methanol treatment. Plants given many applications of aqueous methanol showed symptoms of nutrient deficiency. Supplementation with a source of nitrogen sustained growth, eliminating symptoms of deficiency. Adjustment of carbon/nitrogen ratios was undertaken in the field by decreasing the source of nitrogen in the final application, resulting in early maturation; concomitantly, irrigation requirements were reduced.**

Study of the path of carbon in photosynthesis (1–3) revealed very rapid metabolism of [^{14}C]methanol. From comparison of the relative rates of fixation of [^{14}C]carbon dioxide and [^{14}C]methanol by *Chlorella* and *Scenedesmus* strains, it was concluded that methanol was utilized for sugar and amino acid production fully as rapidly as was carbon dioxide (4). Since both types of early experiments were performed with substrate on a tracer scale, it was not clear that the rates were comparable or what the pathway for methanol conversion to sucrose involved. Subsequent interest in the subject (5) revealed that plants do indeed metabolize methanol rapidly. The conclusion that methanol is readily oxidized to formaldehyde and converted to fructose 6-phosphate has been supported by investigations with bacteria (6) and fungi (7). It was concluded that formaldehyde condensed with pentose 5-phosphate to yield allulose 6-phosphate, which epimerizes to fructose 6-phosphate. [^{14}C]Formaldehyde was also metabolized by algae but, at the tracer level, little sucrose was produced; the activity was extensively bound to protein and not further utilized. It is possible, then, that the first conversion product of methanol, formaldehyde, also binds to proteins at the sites of its production. More recent studies (8, 9) revealed reactions of other metabolic aldehydes with proteins and their consequent structural alteration.

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Treatment of agricultural crops in high solar light intensities and other plants was initiated to determine the economic feasibility of methanol application as a source of fixed carbon or of supplemental methyl groups for pectin production. Rather than merely supporting normal growth, treatment with methanol stimulated growth; its effect on growth far exceeded that expected of a foliar nutrient.‡

MATERIALS AND METHODS

Initial studies were undertaken in the laboratory with methanol added directly to the culture medium of the aquatic colonial alga *Botryococcus braunii* var. Showa (Chlorophyta, Chlorococcales). Standard defined growth media for this alga were supplemented with 3% methanol. Axenic algal cultures were maintained in ambient air with continuous shaking and illuminated with fluorescent light [125 $\mu E \cdot m^{-2} \cdot sec^{-1}$; 1 E (einstein) = 1 mol of photons]. Control cultures without methanol were subjected to identical conditions as well as darkness. After 10 days, the colonies were harvested, dried, and weighed.

Field studies were initiated during the summer on irrigated farm fields in the desert Southwest, Maricopa County, Arizona. Preliminary tests were made in cotton fields, where it was found that a single foliar treatment with 30% methanol and 0.1% surfactant yielded enlarged leaves and taller plants than controls after 2 weeks. Further tests were undertaken in the fall on savoy cabbages, using 20% methanol/0.1% surfactant applications, and these cabbages showed improvements similar to those seen with cotton. After repeated applications of methanol, however, savoy cabbages showed symptoms of nitrogen deficiency. Thereafter, a methanol minimal enhancement medium was formulated containing (g/liter) methanol (100–300), urea (1–15), iron EDTA (0.08), and Triton X-100 (2.5) added to water (pH 6.5–7.0) to appropriate dilution.

Methanol application on winter crops of savoy cabbages did not result in perceptible differences in growth. In attempts to encourage growth, a methanol soluble major and minor nutrient medium was developed and included the following (g/liter): urea (10), urea phosphate (1), potassium acetate (4), sodium isethionate (1), magnesium acetate (2), calcium nitrate (1), iron EDTA (0.08), and minor nutrients (ppm) copper acetate (1), zinc acetate (1), boric acid (2), manganous acetate (1), cobaltous acetate (0.1), and phosphomolybdate (0.01). Application of this major and minor nutrient medium did not result in perceptible differences in growth of most winter crops or shaded plants, but it was later utilized as a 10-fold concentrate to correct for nutrient deficiencies in citrus.

Supplementation of aqueous methanol solutions with 0.1% sodium glutamate or 0.2% glycine increased growth of late

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winter and shaded plants. Glycine/methanol treatment of plants indoors under artificial illumination resulted in foliar damage 48–72 hr afterwards. It was thereby deduced that glycerophosphate (1–6 g/liter) that had supplemented algal cultures might be required. Addition of 0.5% disodium DL-α-glycerophosphate pentahydrate to glycine-enhanced methanol solutions was therefore made to improve turgidity of plants under low-intensity (75–100 $\mu E \cdot m^{-2} \cdot sec^{-1}$) artificial illumination.

All treated and control plants were otherwise given sufficient fertilizers to maintain normal growth; container plants were supplemented with Osmocote 17-6-10 Plus Minors Plant Food for Potting Mixes comprising (percent composition) N (17), P (6), K (10), S (4), Ca (1.5), Mg (1), B (0.02), Cu (0.05), Cu (0.05), Fe (0.4), Mn (0.1), Mo (0.001), and Zn (0.05); crop plants in open farm fields were supplemented with N, P, K, and S farm-grade fertilizers at rates consistent with agriculture for each variety.

Modes of Application. To prevent injury to foliage and to minimize frequency of application in fields, a concentration gradient of methanol in 5% increments was applied to crop plants to establish optimal dose response. Generally, methanol concentrations ≈10% below the established toxicity level elicited a response. For example, a toxicity curve for methanol was established for cotton ranging from 1% to 50% (vol/vol) methanol in pure water. At concentrations above 40% methanol, brown areas and leaf wilt were observed within 10 days. A concentration of 30% methanol in water did not damage cotton leaves, although indentations in some cotton leaves retained 30% methanol for 24 hr or more, and these areas became discolored and brittle. Treatment of cotton fields with 30% methanol minimal enhancement medium was repeated at weekly intervals in two passes. On the final application, 30% methanol without urea source was applied to stimulate maturation of cotton bolls.

Conventional agricultural equipment and machinery were utilized for application of methanol for large areas. For greenhouse application, an overhead mist irrigation system was used. Application of 100% methanol soluble nutrient concentrates to tree trunks and stems was made with a backpack sprayer. Smaller scale tests utilized hand sprayers.

Individual cotton plants were assayed for leaf enlargement by measuring and marking leaf pairs of the same size and at similar positions on separate plants. One cotton leaf was treated with a 30% methanol solution by misting the leaf to wetness, and the other was marked as the control and misted with water. Cotton leaves were treated with three applications and measured for median length and width after 20 days. As another example, individual green cabbage plants were sprayed with a gradient of methanol concentrations to determine toxicity levels. Repeated applications were made when turgidity subsided and treatments were usually spaced 1–2 weeks apart. Field treatments were initiated 2 hr after sunrise and were completed at least 4 hr prior to sunset.

Agriculture. Vegetable and cotton crops were planted in irrigated rows as outdoor commercial crops during 1991. The cotton population was approximately 100,000 plants per hectare. Seeds for field crops were from commercial sources in Maricopa County, AZ, as follows: savoy cabbage (*Brassica oleracea capitata*), 'Savoy King,' Sakata Seed America; green cabbage (*Brassica oleracea capitata*), 'Head Start,' hybrid, Asgrow Seed; short staple cotton (*Gossypium hirsutum*), 'Deltapine 90,' Delta & Pine Land Company; durum wheat reva (*Triticum durum*), Arizona origin, Borden Pasta Group; barley (*Hordeum vulgare*), Arizona origin, Salt River Seed and Soybean Company; canning tomato (*Lycopersicon esculentum*), 'Genoa,' Northrup King.

Watermelons (*Citrullus vulgaris*, 'Calsweet,' Asgrow, Maricopa County, AZ) were planted in sandy loam soil. Test rows were alternated with control rows; all plants otherwise were given identical agricultural treatment. Rows were spaced 2 m apart and vines were thinned to 1- to 1.5-m spacing between plants. Watermelons are often damaged by foliar sprays; the dose–response of watermelon foliage showed toxicity at 12% methanol, but addition of glycine allowed increased methanol concentrations without symptomatic foliar injury. A single spray application of 16% methanol minimum enhancement medium plus 0.1% glycine was made on vines by tractor at a rate of 200 liters/ha. The foliar application was made at noon on a clear sunny day in May, ≈4 months after planting, when vines completely covered the width of the raised row beds. Fields were irrigated regularly as needed. Only fully ripened melon fruit was harvested into tared trucks. The first harvest was made 40 days after treatment with methanol, followed by three more harvests within 75 days of treatment as melons ripened. Melons were weighed on calibrated farm scales with fruit tonnage calculated by subtracting the tare weight of the truck from the total. Test rows covered a total area of 1 ha. Following harvest and measurement, edible plants that were treated with methanol were destroyed by plowing them 30–50 cm underground.

Horticulture. In spring, commercial greenhouses (Maricopa County, AZ) were stocked with 3000 hybrid tea rose (*Rosa* spp.) cultivars cultured in 8- to 12-liter plastic containers for growth from bare root stock to bud and bloom in potting medium composed of 90% bark, 5% river sand, and 5% topsoil and given sufficient fertilizers to maintain normal growth. Controls in an identical greenhouse were given water through the mist irrigation system when treated plants were given 10% methanol minimal enhancement medium.

Lemon (*Citrus limon*), sour orange (*Citrus aurantium*), grapefruit (*Citrus paradisi*), *Eucalyptus microfica*, *Olea europaea*, *Phoenix canariensis*, *Washingtonia robusta*, *Pinus eldarica*, and *Pinus halepensis* trees were treated by spraying stems or foliage with methanol solutions.

Container seed starts of wheat, barley, and tomato were germinated in 72-well plastic tree flats with 90% bark/5% river sand/5% topsoil and fertilizer. Wheat was subjected to direct sunlight and water stress by eliminating two consecutive irrigation cycles and then resuming normal irrigation cycles. Wheat was treated with 20% methanol and soluble major and minor nutrients 2 days prior to water stress. At maturity, 50 seed heads including the rachis, seed, and chaff were weighed and seeds per head were counted for both control and treated plants.

Barley (*Hordeum vulgare*) was tested for effects of low light intensity by shading with 85% blockage mesh and given three spray treatments during the 2-week test period. Six sets of barley were prepared: two for direct sunlight, two for shade, and two for shade with sodium glutamate (1 g/liter in methanol minimal enhancement medium). Half of the sets were left untreated as the control and the other sets were treated with test solutions. In a repeat of the test and to confirm that sodium was not responsible for growth responses, glycine (2 g/liter) was substituted for the sodium salt of glutamate. Increased turgidity was determined by measuring the angles of pretreatment and posttreatment positions of barley leaves.

For confirmation of yield increases given a standardized glycine medium, plants were treated with 20% methanol minimal enhancement medium supplemented with glycine (2 g/liter) during the cool late winter from 1 March to 1 April 1992. Test plants were treated three times under cloudy weather conditions. The following cultivars were treated: 'Ichiban' eggplant, 'Genoa' tomato, and 'Sequoia' strawberry. Plants were 5–10 cm tall at the start of weekly treatments. Harvest was undertaken by cutting the entire shoot at the base. Live weights of the shoots and individual leaves were recorded for controls and for methanol/glycine-treated plants.

For a general houseplant formulation, 0.1% glycine in 10% methanol minimal enhancement medium was supplemented with 0.5% disodium glycerophosphate pentahydrate and manually applied to foliage as a fine mist. The glycerophosphate solution was applied to *Chrysanthemum indicum*, *Dieffenbachia sequine*, *Syngonium podophyllum*, *Scindapsus aureus*, *Ficus elastica*, and *Coleus blumei*. Plants were observed for increases in turgidity and signs of toxicity for 2 weeks under artificial illumination.

During autumn in open fields, 20% methanol was sprayed on foliage of plants with C_4 metabolism: corn (*Zea mays*, 'Sweetie 82,' Sun Seeds, Maricopa County, AZ), sorghum (*Sorghum vulgare*), bermuda grass (*Cynodon dactylon*), and johnson grass (*Sorghum halepense*). Two or more foliar applications were made 1 week apart and plants were observed for 1 month. Corn leaf lengths and number of cobs were measured on tagged methanol-treated plants and matched controls in adjacent rows within a 10-acre field (1 acre = 0.4047 ha). Sucrose content of tomato fruit was measured in the field with an American Optical refractometer.

The colonial alga *Botryococcus braunii* var. Showa (Chlorophyta, Chlorococcales) (10) (type specimen deposited in the University of California, Berkeley, with accession no. UC147504) produces hydrocarbons that can be used as transport fuels (11). An axenic isolate of this variety was prepared by Ralph A. Lewin (Scripps Institution of Oceanography). Voucher specimens of treated and control savoy cabbages were deposited with lot no. UCB11012.

RESULTS

Initial tests undertaken on the colonial alga *Botryococcus braunii* cultured for 10 days with addition of methanol showed approximately double the average dry weight yield (116 mg) as compared to average dry weight yields of controls (59 mg) supplemented with carbon dioxide. In ambient atmosphere, average dry weight yields of methanol-treated cutures (52 mg) were greater than controls (45 mg). Neither treated nor control cultures showed growth in the dark during the 10-day period. From these early tests, methanol was shown to be the active component and photosynthesis was required.

When exposed to sunlight, plants showed rapid responses to methanol just below toxicity levels. Toxicity levels of methanol varied according to anatomical location of application and variety of plant. Generally, stems withstood the highest concentrations; 80–100% methanol was applied directly to trunk sections of pine (*Pinus eldarica* and *Pinus halepensis*), palm (*Phoenix canariensis* and *Washingtonia robusta*), eucalyptus (*Eucalyptus microfica*), lemon (*Citrus limon*), sour orange (*Citrus aurantium*), grapefruit (*Citrus paradisi*), and olive (*Olea europaea*) trees with no observable damage. When applied to *Pinus eldarica* stems that had been trimmed 3 months earlier, 90% methanol caused sap to run out of old wounds within 12 hr. Penetration through woody bark of the pine branch was immediate and translocation was clearly evident from the new sap emergent upstream from treatment with methanol. Germlings of *Washingtonia robusta* palms were sprayed with 50% methanol minimal enhancement medium once per month for 6 months, and five whole shoots of untreated controls averaged 15 g each while five treated palm shoots averaged 26 g each. Responses to a gradient of methanol concentrations on tomato (*Lycopersicon esculentum*) showed increased damage to leaf margins from 20–40% methanol and no phytotoxicity at 10% methanol within 4–10 days. Under direct sunlight, gains in growth of tomato plants treated three times with 10% methanol minimal enhancement medium were visible over controls within 2 weeks of treatment, controls showing 9–10 internodes and treated tomato plants showing 12–16 internodes. Treated tomato plants had leaves and stems which were 25–50% thicker in diameter than controls. Fruit development on treated tomato plants commenced 5–10 days earlier than controls. Fruit of methanol-treated tomato plants averaged 10–12% greater sugar content than untreated controls after 1 month.

Foliar requirements for methanol differed widely. For example, 50% methanol was applied to palm and eucalyptus leaves, but eggplant was treated with 10% methanol. Significant differences in optimal methanol concentrations for foliage were observed at the varietal level, exemplified by savoy cabbage at 20% methanol and green cabbage at 50% methanol concentration in water. Foliar applications far below established toxicity levels necessitated repeated applications to elicit rapid growth responses similar to applications made near the toxicity levels. For example, at 20% methanol concentration, green cabbage required three to six repeated applications to show response similar to a single 50% methanol application. Untreated control cabbages were similar in size to cabbages treated with one application of 20% methanol, but cabbages treated repeatedly with 20% methanol or once with 50% methanol grew to approximately twice the size of controls in 4 weeks. With foliar treatment under direct noon sunlight, increased turgidity was observed within 2 hr of treatment with methanol. Increased turgidity in treated plants was particularly evident between irrigation cycles and in the afternoon, when control plants wilted under direct sunlight. Treated plants stood erect and vigorous during periods when controls were water-stressed. Under high noon direct sunlight, for example, foliar application of 30% methanol on cotton resulted in increased leaf turgidity within 4 hr and ≈15% increased growth in height over untreated controls within 2 weeks. During 1990, when 45–50°C weather was experienced, treated cotton plants remained turgid while the rest of the crop wilted at peak afternoon temperatures. In a 23-ha field treated twice 12 weeks prior to harvest of cotton, fruit matured ≈2 weeks earlier than untreated fields. This early maturation allowed irrigation to be terminated 15 days early.

Savoy cabbages were treated under direct sunlight with 20% methanol. During a week when temperature maxima were above 40°C, treated savoy cabbages remained turgid while controls wilted. During the fall, savoy cabbages treated with a single application of methanol showed an ≈50% increase in vegetative growth over controls after 2 weeks, with larger, thicker, and more numerous leaves. Savoy cabbages treated with multiple applications of methanol showed chlorosis and stunted growth after the fifth application; therefore, nutrient-supplemented solutions containing urea and chelated iron were utilized to sustain growth. After 4 weeks and three treatments with 20% methanol minimal enhancement medium, treated cabbages were as much as twice the size of controls. In a long-term test for 60 days and with 10 applications of 20% methanol minimal enhancement medium, 10 treated savoy cabbages averaged 3.5–4.0 kg per individual head while 10 controls averaged 2.0–2.5 kg per individual head. In a practical field test of rate of maturation, 100 savoy cabbage plants were treated five times during the fall season of 1991 with 20% methanol minimal enhancement medium. Harvest was undertaken by uninformed field hands who selected only those cabbage heads that were greater than 1–1.5 kg each. Treated savoy cabbages matured more evenly and earlier than 100 untreated controls. Of the 100 treated savoy cabbage plants, 75 heads were harvested on first pick. In contrast, 16% of the untreated savoy cabbage plants were harvested on first pick. Largest heads were found in the treated areas and weighed 3.5–4.0 kg. The largest heads found in control rows were 2.5–3.0 kg.

Further cabbage assays were undertaken on winter sets during short days when cloud cover and rain was frequent.

Under these cool, wet, low-light intensity conditions of winter, differences between treated and control cabbages were generally imperceptible.

Watermelon vines treated with methanol at noon showed highly turgid vines with standing foliage prior to sunset of the same day. Controls showed distinctly less vigor and, characteristic of normal appearances, foliage was generally oriented parallel to the plane of the ground rather than standing erect. Watermelons showed an ≈50% increased fruit yield over controls attributable to prior treatment with methanol. After 40 days, the first harvest following treatment with methanol yielded 37 t/ha, where control rows yielded 25 t/ha. The ensuing harvests of watermelons yielded a grand total of 74 t/ha from the test section and 54 t/ha from controls. The seasonal total showed a 36% increase in yield of treated melons over controls. The cost of methanol per application was 1% of the economic gain achieved by improved fruit yield.

Our preliminary tests on roses with high concentrations of iron showed that iron EDTA at 0.9 g/liter in methanol was phytotoxic, but 0.08 g/liter in methanol was the maximum concentration tolerated by young rose foliage. A very high C/N ratio was achieved in the final foliar application because no urea was added. When treated with methanol, cultivars Rotary Rose, Paul Harris, Miss All-American Beauty, Blue Girl, Tiffany, Mr. Lincoln, John F. Kennedy, Joseph's Coat, Peace, Lowell Thomas, and Queen Elizabeth grew to bud and bloom within 62 days of placement in the greenhouse. Treated cultivars Angel Face, First Prize, and Tropicana required ≈70 days to reach bud and bloom. In the control greenhouse, all varieties required 75–80 days to achieve bud and bloom maturity. Treated roses showed fuller foliage and blooms than controls. 'Miss All-American Beauty' flowers, for example, averaged 26 g compared with 18 g for controls. At the time of first bloom, treated 'Paul Harris' plants averaged eight fully opened flowers. Controls later averaged four fully opened flowers. Plants remained healthy and pest-free.

Durum wheat (*Triticum durum*) treated under direct sunlight with three applications of 20% methanol with soluble nutrients was erect and turgid during water stress while controls wilted for ≈2 hr each afternoon after elimination of the second irrigation cycle. Treated wheat foliage averaged more than 50% greater in length and 35% greater in width than blades of untreated controls 45 days after planting. At harvest, the culms of methanol-treated wheat averaged twice the number of seeds and weight of the corresponding controls.

Methanol was applied to short staple cotton (*Gossypium hirsutum*) planted out-of-doors in irrigated rows as a commercial crop from June through August. Within 2 weeks of treatment with 30% methanol minimal enhancement medium, cotton plants showed greater turgidity and had larger leaves than controls. Treatment with methanol stimulated development of cotton leaves to 20% or greater surface area and 25–50% greater thickness than controls in 2 to 3 weeks. Greatest leaf increase was observed in the upper canopy and least improvement was observed in lowest leaves. Individual cotton plants received ≈0.5 ml of methanol per plant. Treated cotton plants required irrigation repetitions 9 days apart when control plants required 7-day irrigation cycles during a 20- to 30-day period following methanol application. Fewer irrigation cycles on treated cotton fields in 1992 reduced the normal seasonal total water orders (Maricopa Water District) by half, from 122×10^5 liters/ha to 60×10^5 liters/ha.

Correction of nutrient deficiency in citrus was made by application of a 10-fold concentrated methanol-soluble major and minor nutrient enhancement medium. At 3-month intervals, the 100% methanol nutrient concentrate was sprayed on the trunks of three nutrient-deficient grapefruit (*Citrus paradisi*) trees. At the beginning of the new growth season, no foliar symptoms of nutrient deficiency were observed in new foliage of treated trees, whereas the controls were unimproved.

After 2 weeks under direct sunlight conditions, barley treated with methanol minimal enhancement medium showed an ≈50% increase in vegetative growth over controls. Under subdued light, controls were slightly etiolated and averaged 10.5 cm in height. Barley treated with methanol minimal enhancement medium under low light averaged 6.3 cm in height and had wilted brown leaf tips. With the addition of glutamate to minimal enhancement methanol medium, barley plants averaged 12.3 cm under low light conditions. In the repeat of the test with glycine substituted for glutamate, barley plants showed similar improvement of growth in the shade. Barley plants that were treated with glycine and methanol showed turgidity increases within 30 min under direct sunlight and after several hours in the shade. Wilted blades of treated plants rose 25° above their pretreatment positions.

The detoxifying characteristics of glycine observed in tests with shaded barley implied that higher concentrations of methanol might be applied without injury. With the addition of glycine to methanol solutions, rose and tomato showed no phytotoxicity from 20% methanol solutions except in areas of accumulated solution. Without glycine, rose and tomato plants developed brittle brown leaf margins after treatment with 20% methanol.

Based on our observation that the addition of glycine improved plant response under shaded sunlight and that glycine reduced toxicity of methanol, a standard solution for use during cloudy weather or with indirect sunlight was formulated as follows: 20% methanol, 0.1% urea, 0.1% urea phosphate, 0.1% glycine, 0.05% Triton X-100, and water. This standard formulation was applied manually once per week for 3 weeks to eggplant, strawberry, and tomato plants cultured in containers out-of-doors with the improvements shown in Table 1.

After treatment with methanol/glycine formulations, plants required exposure to sunlight. When maintained in the shade, methanol/glycine-treated rose foliage developed irregularly shaped black areas. No foliar damage was observed when plants were exposed to sunlight within 24 hr of methanol/glycine treatments. Based on the posttreatment requirement for photosynthesis to detoxify products of methanol/glycine treatment under low light conditions, glycerophosphate was added. A 20% methanol/glycine/glycerophosphate solution was applied to rose foliage indoors. As controls, separate rose foliage was misted with methanol/glycine, methanol, or water. After 5 days, the water-misted controls were slightly etiolated and showed elongated internodes. Control rose plants showed water stress, with all flowers wilting. Methanol controls showed extensive phyto-

Table 1. Growth and yields of methanol-treated plants

Plant	Days from treatment	Yields*	Leaves†	Growth, % improved
Botryococcus	10			100
Tomato	30	65; 41		50
Strawberry	30	28; 17	7; 4.1 (5; 2.6)	60
Eggplant	30	57; 35	17; 5.6 (7; 4.4)	60
Cotton	30			50
Savoy cabbage	60			50
Wheat (fruit yield)	60			100
Rose	45			40
Palm	180			70
Melon (fruit yield)	75			36

*Weights (g) of entire treated and control plants.
†Average number of leaves (and weights of largest leaf, g) for treated and control plants.

toxicity, with dead brittle brown whole leaves and margins, whereas methanol/glycine controls showed irregular black areas on leaves. Foliage treated with methanol/glycine/glycerophosphate was healthy, green, and turgid with blooms erect and developing to fullness.

Foliar sprays of 10% methanol/glycine/glycerophosphate on *Chrysanthemum indicum*, *Dieffenbachia sequine*, *Syngonium podophyllum*, and *Scindapsus aureus* produced increased turgidity and healthful growth indoors with artificial illumination (75–100 $\mu E \cdot m^{-2} \cdot sec^{-1}$) over a period of 1 week. Increased turgidity of subapical leaves of *Ficus elastica* was evident within 2 hr. Daily treatments of *Coleus blumei* with 20% methanol/glycine/glycerophosphate resulted in plants with average individual weights of 43 g compared with water-misted controls with average individual weights of 36 g after 10 days.

No positive growth effects were observed after application of any concentration of methanol to plants with C_4 metabolism: corn, sorghum, bermuda grass, or johnson grass. Five foliar applications of 20% methanol caused minor leaf damage and no greater leaf expansion or earlier maturation than controls. Methanol-treated corn showed no differences in fruit or vegetative measurements and showed linear brown areas along median leaf veins, as well as undulating leaf texture.

DISCUSSION

The immediate plant response to methanol treatment under direct sunlight was a visibly perceptible increase in turgidity, generally indicative of opened stomata. Turgid guard cells and open stomata permit increased carbon dioxide assimilation; concomitantly the rate of transpiration is increased (e.g., ref. 12), a possible factor contributing to reduction of water stress. Applications of quantities of methanol that are minute compared to the volumes of water for irrigation necessary to maintain the same degree of plant turgidity reduce irrigation requirements. Our field results showing increased number of days between irrigation cycles in cotton fields (73 and 236 ha) and reduced agricultural water delivery support this conclusion. Our observed improved growth rates offer additional water savings by early harvest of crops.

Increased growth responses and increased turgidity observed after methanol application to foliage of C_3 plants indicate that wetting agents effectively aid penetration. Early flower and fruit development in rose, cabbage, cotton, tomato, and wheat treated with methanol demonstrate that manipulation of C/N ratios for control of maturation processes presents considerable potential for enhancing production in field environments. This manipulation of C/N ratios is similar to control of sugar or nitrogenous substrates in plant tissue culture for specific plant processes (e.g., refs. 13–15). Symptoms of nutrient deficiency in plants (16) that were treated frequently with methanol provided a rationale for supplementation with other major and minor nutrients. Sustained growth improvement in crop plants was observed after supplementation with urea (providing N) and chelated iron. Application and uptake of soluble nutrients by the foliar or stem routes demonstrated the dual benefits of nutrient-deficiency alleviation and methanol stimulation.

We observed substantial increases in yields of all C_3 plants treated with nutrient-supplemented methanol and maintained under direct sunlight. Shaded plants showed toxicity responses to methanol, leading us to surmise that the photosystem was required for metabolism of methanol. Our observation that addition of glycine eliminated methanol toxicity, actually allowing increased concentrations of methanol to be applied without injury, indicated the involvement of photorespiration. Previous studies with radiolabeled glutamate (17) demonstrated increases in glycine after addition of glutamate, indicating that photorespiratory metabolism was not inhibited. Lack of response to methanol by C_4 plants, which do not photorespire significantly, appeared consistent with the indication that metabolism of methanol required photorespiration, common to C_3 plants under direct sunlight. The high turgidity observed in field plants soon after application of methanol suggested rapid metabolism of carbon to sugar. Our observations showed that detoxification of methanol and its oxidation product formaldehyde required both glycine and posttreatment exposure to bright light or glycerophosphate and light, leading us to conclude that both photorespiration and photosynthesis are involved.

Concurrent studies by Benson and Nonomura (18) showed that the ratio of sucrose to glycolate is greatly increased by prior exposure to methanol, suggesting that long-term stimulation of growth may be related to inhibition of photorespiration. Overall, our studies suggest that plants respond to high concentrations of methanol in two or more stages, first utilizing photorespiratory and other available metabolic pathways for detoxification and, thereafter, activating a mechanism that improves carbon fixation. Integration of these photometabolic events was demonstrated in our studies of watermelon crops, where foliar application of 32 liters of methanol improved fruit yield by 12 t over controls.

We have demonstrated utilization of conventional technology for foliar application of methanol from laboratory to agriculture. Increases in yields have been consistently significant (Table 1). We conclude that the proper application of methanol provides the potential to reduce water requirements and to improve crop yields.

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