The Role of Methanol in Promoting Plant Growth: A Current Evaluation

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The biochemical responses of plants to methanol have become subjects of controversy since Nonomura and Benson (1992a, 1992b) reported remarkable growth enhancement of C₃ plants treated with methanol. Despite the authors' numerous positive findings, other researchers have had mixed results. Although some positive responses have been reported (Devlin et al., 1994; Hemming et al., 1995; Li et al., 1995; Nishio et al., 1994; Valenzuela et al., 1994), most publications report no evidence of gain in biomass in treated plants (Hartz et al., 1994; Mauney and Gerik, 1994; McGiffen et al., 1995; Mitchell et al., 1994). These contradictory results have left the efficacy of methanol treatments unresolved.

Methanol metabolism in plants is poorly understood. Methanol is a naturally occurring, volatile organic compound emitted from the leaves of many plant species (MacDonald and Fall, 1993; Nemecek-Marshall et al., 1995), but the origin of emission is uncertain. Interestingly, leaves typically harbor methylotrophic bacteria capable of metabolizing methanol (Corpe and Rheem, 1989; Holland and Polacco, 1994), and these bacteria may be associated with leaf surface methanol emission. Methylotrophic yeast is also capable of metabolizing methanol. Since microorganisms have diverse pathways of methanol metabolism, it is not surprising that plants have several biochemical pathways for converting low levels of methanol into C₁ metabolites (Cossins, 1964, 1980; Doman and Romanova, 1962).

In this report, we discuss key similarities between pathways of methanol assimilation in microbes and those proposed in higher plants. We then review the findings on the effects of methanol on plant growth and development.

METHANOL ASSIMILATION BY METHYLOTROPHIC MICROORGANISMS

Methanol metabolism in methylotrophic microorganisms has been the subject of intense research aimed at developing sources of single-cell protein from abundantly available methane and methanol (for a review see Murrell and Dalton, 1992). The reaction site in methylotrophic yeast is in peroxisomes, where methanol is oxidized to formaldehyde by alcohol oxidase. The ultimate fate of the methanol, either linear oxidation to CO₂ or cyclic assimilation of the methanol carbon into carbohydrate, is tightly controlled, thus preventing toxicity to the cell. Bacteria use methanol by other distinct pathways (Anthony, 1982), and methylotrophic yeast and bacteria differ in the initial catabolism of methanol oxidation (Alawadhi et al., 1988; Babel and van Verseveld, 1987; Dijkhuizen et al., 1992; Duine et al., 1984; Jongejan and Duine, 1989; Quilter and Jones, 1984).

In both methylotrophic yeast and bacteria, the oxidation of formaldehyde and formic acid to CO₂ generates reducing equivalents. Formaldehyde is oxidized to formic acid by NAD⁺-linked aldehyde dehydrogenases that typically differ in the required cofactors, including tetrahydrofolate (Attwood and Quayle, 1984) and reduced glutathione (Johnson and Quayle, 1964). In most organisms, catalysis of formate oxidation is by soluble NAD⁺-requiring formate dehydrogenases (Karzanov et al., 1989).

One of the methylotrophic pathways of interest in understanding putative pathways for methanol use by higher plants is the bacterial serine pathway (Quayle, 1972). This pathway (Fig. 1) uses glycine as the acceptor of the oxidized methanol intermediate, in this case a form of hydroxymethyltetrahydrofolate. The resulting serine molecule is ultimately converted, in combination with an additional molecule of CO₂, to C₄ metabolites (Quayle, 1980). In this way, C₁ substrates couple with glycine to function as carbon and energy sources for cell metabolism. The coupling avoids the toxicity resulting from formaldehyde crosslinking with cellular proteins.

METHANOL ASSIMILATION IN PLANTS

In contrast to the definitive characterization of the biochemical pathways of methanol metabolism in methylotrophic yeast and bac-

teria, very little is known of the fate of methanol in plant tissue. Few attempts have been made to characterize the assimilation of reduced C₁ carbon sources (i.e., formate, formaldehyde, and methanol). Early work by Doman and Romanova (1962) indicated that these C₁-carbon sources are initially converted in leaves to CO₂, which is rapidly assimilated during photosynthesis. Assimilation rates in darkness are several times slower. However, Cossins (1964) showed that, without light, plants readily metabolize low levels of [14C]-methanol to ¹⁴CO₂. Additional label from [14C]-methanol was incorporated into organic acids, sugars, and amino acids (primarily serine and methionine).

The high levels of labeled formate and CO₂ produced during metabolism of methanol in darkness indicated that the main pathway initially involved methanol oxidation to formaldehyde (Fig. 2). Aldehyde and formate dehydrogenases in plant tissue rapidly catalyze formaldehyde and formate oxidation, respectively (Giese et al., 1994; Halliwell, 1974; Oliver, 1981). In this way, plants are similar to unicellular methylotrophs in the ability to drive methanol oxidation to CO₂ and thus avoid the toxicity associated with formaldehyde accumulation (Fig. 2). Cossins (1980) speculated that label was partitioned to the other cellular components through the transfer of an active formyl species, complexed with tetrahydrofolate, to glycine and homocysteine, which produce serine and methionine, respectively (Fig. 2). This process was later shown to occur in the bacterial serine pathway (Fig. 1; Anthony, 1975; Quayle 1972, 1980).

Related studies of ethanol metabolism showed ethanol oxidation in many plants (Cossins, 1978) but only limited metabolism to CO₂. Cells of carrot [*Daucus carota* subsp. *sativus* (Hoffm.) Arcang.] grown in suspension culture used alcohol dehydrogenase to convert ethanol slowly to acetaldehyde (Perata and Alpi, 1991a). Negligible acetaldehyde oxidation and assimilation resulted in toxic accumulation of acetaldehyde (Perata and Alpi, 1991b; Perata et al., 1992). Thus, alcohol oxidation in plant tissue that cannot rapidly convert aldehydes via oxidation or assimilation results in aldehyde toxicity and cell inactivation.

However, in leaves undergoing rapid photosynthesis, light-regulated metabolism and the physiologic features of photosynthesizing plant tissue may be sufficiently different so as to alter the way plant cells metabolize the

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Fig. 1. Bacterial serine pathway (Quayle, 1980). F1D = formaldehyde dehydrogenase; F2D = formate dehydrogenase; THF = tetrahydrofolate.

Fig. 2. Methanol assimilation and linear oxidation to CO_2 in plants (Cossins, 1964). F1D = formaldehyde dehydrogenase; F2D = formate dehydrogenase.

intermediates of methanol oxidation. This difference is the basis of the proposed mode of action of methanol in promoting plant growth outlined in the next section.

METHANOL IN PLANTS: A LINK WITH PHOTORESPIRATION

The apparent rapid assimilation of methanol into photosynthesizing plant tissue and the ensuing increases in plant growth after the exposure of plants to methanol were first reported by Nonomura and Benson (1992a, 1992b). Applications of methanol to some C_3

plant species during periods of elevated photorespiration resulted in increased plant turgidity, biomass, and rates of development. Increased growth occurred in tomato (*Lycopersicon esculentum* Mill.), 50%; strawberry (*Fragaria xananassa* Duchesne), 60%; eggplant (*Solanum melongena* L.), 60%; cotton (*Gossypium hirsutum* L.), 50%; savoy cabbage (*Brassica oleracea* L. Capitata group), 50%; wheat (*Triticum aestivum* L.), 100%; rose (*Rosa* spp.), 40%; palm (*Washingtonia robusta* H. Wendl.), 70%; and watermelon [*Citrullus lanatus* (Thumb.) Matsum & Nakai], 36%. Dramatically different water use was

indicated by the ability of the methanol-treated plants to withstand wilting. Importantly, C_4 plants given methanol under the same conditions showed none of these effects.

The clear distinctions between C_3 and C_4 plants in the effect of methanol were attributed to the inhibition of photorespiration during methanol assimilation (Nonomura and Benson, 1992a, 1992b). This proposal was supported by the finding that increased amounts of methanol could be applied without toxic effects when glycine was included in the spray solution. Nonomura and Benson proposed that glycine produced during photorespiration was essential for methanol assimilation (Fig. 3). Plants not undergoing photorespiration could not assimilate methanol and showed toxicity symptoms following methanol applications. Glycine acts similarly in the assimilation pathway (Fig. 2), outlined earlier by Cossins (1964), and in the microbial serine pathway (Fig. 1).

Also important to methanol's proposed mode of action in photosynthesizing C_3 plants was the ensuing inhibition of photorespiration, presumably through sharp increases in the leaf internal CO_2 concentration. Such increases would shift the catalysis of the ribulose biphosphate carboxylase away from the O_2 concentration-dependent oxidation of ribulose diphosphate. Hence, the increased biomass of the methanol-treated C_3 plants was attributed to 1) the use of the methanol as a direct carbon source via serine biosynthesis and 2) decreased carbon loss from photorespiration.

Nonomura and Benson (1992a, 1992b) proposed that the metabolism of methanol lessened water use in plants. Metabolism of methanol to sugars would change leaf osmotic potential, resulting in increased turgor and stomatal conductance. Keeping the stomata open would increase the assimilation rate and, subsequently, plant growth. The accelerated growth rate resulted in earlier maturation and thus less need for irrigation.

Supporting these hypotheses was the recent finding of Nishio et al. (1994) that applying methanol to hydroponically grown spinach (*Spinacia oleracea* L.) increased CO_2 uptake by $\approx 20\%$; this effect persisted for at least 14 days. As well, leaves of methanol-treated sugar beet (*Beta vulgaris* L.) plants had higher internal CO_2 levels. Turgidity increased in soybean [*Glycine max* (L.) Marr.] plants under severe water deficit that were treated with a 15% solution of methanol.

Several C_3 solanaceous plants exposed to methanol under laboratory conditions displayed a systemic response in respiration rate (Hemming et al., 1995). Doses of methanol that produced maximal responses increased plant respiratory rates by almost 50%. Tomato plants grown out of doors had sustained increases in metabolic heat-production rates but not growth rates when exposed periodically to various doses of methanol. Two cultivars of maize (*Zea mays* L.), a C_4 species, failed to respond to methanol applications.

Applications of methanol to various C_3 plants increased plant growth (Devlin et al., 1994; Li et al., 1995; Rowe et al., 1994). Results of greenhouse experiments showed

Fig. 3. Proposed mechanisms of growth enhancement of methanol-treated C_3 plants.

increased growth of methanol-treated wheat, radish (*Raphanus sativus* L.) and pea (*Pisum sativum* L.) but not maize (Devlin et al., 1994). Applications of methanol significantly increased yield but not chlorophyll content or net photosynthetic rate in soybean (Li et al., 1995). Rowe et al. (1994) applied 0% to 20% solutions of methanol or ethanol without surfactants or nutrients to the foliage and roots of greenhouse-grown tomato. A 5% solution of methanol applied to roots severely decreased shoot growth, and higher concentrations killed the plants. However, applications to leaves increased the stem weight, and fresh and dry leaf weight by up to 19%, with methanol having a somewhat greater effect than ethanol.

IRREPRODUCIBILITY OF REPORTS OF METHANOL AS A GROWTH PROMOTER

Several attempts to reproduce findings of methanol-induced plant growth have failed. McGiffen et al. (1995) closely duplicated the experimental design of Devlin et al. (1994) but found that methanol applications correlated with injury rather than yield increase. The authors also conducted field experiments on several crops in climates ranging from coastal to desert. Lemons (*Citrus limon* L.), carrots, and turf [creeping bent grass (*Agrostis palustris* Huds.)] were unaffected by methanol, but desert-grown lettuce (*Lactuca sativa* L.) showed pronounced injury after only one application of a 30% solution. Hartz et al. (1994) conducted similar experiments and was unable to demonstrate methanol-induced growth promotion in melons [*Cucumis melo* L. and *Citrullus lanatus* (Thunb.)] or tomato. Albrecht et al. (1995) found that applications of methanol at concentrations up to 90% to winter wheat, spring barley (*Hordeum vulgare* L.), and pea had no effect on yield, canopy or leaf temperature, stomatal conductance, or leaf weight. Crowe et al. (1994) found that vegetative growth, number of tillers, and seed yield of Kentucky bluegrass (*Poa pratensis* L.) declined following foliar applications of methanol. Foliar applications of methanol had no effect on tuber yield, size, or quality in potato (*Solanum tuberosum* L.) (Feibert et al., 1995).

Idso et al. (1995) applied solutions of 40% methanol to sour orange (*Citrus aurantium* L.)

trees grown in open chambers that maintained either ambient ($400 \mu\text{mol}\cdot\text{mol}^{-1}$) or enriched CO_2 ($700 \mu\text{mol}\cdot\text{mol}^{-1}$). Enrichment of CO_2 raised the upper-limit leaf temperature for positive net photosynthesis. However, even when photorespiration was high enough to result in a negative assimilation rate, methanol had no effect on photosynthesis in either ambient or enriched CO_2 . Methanol application lowered leaf temperature. If photosynthetic rates were not corrected for leaf temperature changes, methanol had no effect on the orange trees in ambient CO_2 . The methanol treatment raised photosynthetic rates 52% in the enriched environment. The authors concluded that the rise in photosynthetic rates was solely due to cooling the leaves to a temperature more optimal for photosynthesis, not to methanol metabolism in the oranges.

The effect of methanol on yield and photosynthesis was intensely investigated in field and laboratory tests in states in the cotton belt (Mauney and Gerik, 1994). Methanol had little effect on yield or photosynthesis. The few scattered exceptions included a 10% increase in photosynthetic rate and stomatal conductance at locations in California and Texas. An increase in harvestable yield or biomass was attributable to methanol only in the California location. A few locations exhibited positive effects such as increased fiber strength (Thrall, Texas), increased leaf area and mainstem nodes (Corpus Christi, Texas), and decreased infestations of whitefly (spp. not reported; Phoenix). Because yield in cotton is directly correlated with photorespiration, the lack of yield response to methanol led the authors to conclude that photorespiration was not affected.

van Iersal et al. (1995) explored the effect of methanol on photosynthesis in cotton in field experiments in Mississippi, Arkansas, and North Carolina and found that foliar applications had no effect on yield, timing, or leaf and floral development. Methanol delayed maturity of bolls but did not affect any other yield component. Water potential, and transpiration and photosynthetic rates were measured between 2 h and 6 days after applications of methanol applications to single leaves and whole canopies. All variables were unaffected by foliar applications of methanol and nutrients, or nutrients without methanol. The

CO_2 compensation point, an indicator of photorespiration, was similar in all treatments. The authors concluded that applications of methanol did not affect gross photosynthesis and photorespiration and, thus, water-use efficiency. Leaf water potential and its two components, osmotic and turgor pressure, were also unaffected.

In calculating the carbon input from foliar-applied methanol, van Iersal et al. (1995) demonstrated that eight applications of 30% methanol to a cotton crop were equivalent to 166 kg carbon/ha. If 100% of the carbon was absorbed by the leaves and allocated to the fruit, and 27% of each boll's weight is lint, yield could have increased $47 \text{ kg}\cdot\text{ha}^{-1}$, or 4%, which is well below the 30% yield increase reported by Nonomura and Benson (1992a). However, 4% is overly optimistic, because it assumes that all of the methanol was absorbed and translocated directly for fruit growth. Since methanol is volatile, much of it likely would have evaporated before it was absorbed by the leaves.

Faver and Gerik (1996) observed slight increases in assimilation rate and stomatal conductance following foliar applications of methanol to cotton. However, if metabolism of methanol in leaves had inhibited photorespiration, the rise in assimilation rate would not have been associated with increased internal CO_2 concentration. The inhibition of photorespiration would have lowered internal CO_2 concentration as more CO_2 was consumed with the rise in net assimilation. Decreased photorespiration would also result in increased leaf water-use efficiency, since there would be an increase in carbon assimilation, with little change in transpiration. Instead, the authors found an increase in assimilation rate and internal CO_2 concentration, with no change in growth, phenology, water use, or any other characteristic. They hypothesized that methanol may be metabolized by microbes on leaf surfaces that generate ATP and NADH, which increase the turgidity of guard cells and thus stomatal conductance, internal CO_2 concentration, and CO_2 assimilation rate.

CONCLUSION

The overwhelming evidence from the scientific literature is that methanol is not a reliable stimulant of crop productivity. Although the methods have not been consistent across all of the studies, investigations that duplicated Nonomura and Benson's (1992a, 1992b) procedures failed to find consistent increases in crop yield (Hartz et al., 1994; Mauney and Gerik, 1994; McGiffen et al., 1995). Additional field experiments that simply repeat Nonomura and Benson's (1992a) original protocol are unlikely to provide new insight. The foliar application of methanol will unlikely become a practical yield enhancer until a plausible mechanism of increased productivity through methanol's effect on plant biochemistry has been discovered.

Yet the substantial enhancement in plant growth reported in a few cases and the intriguing possibilities of C_1 -tetrahydrofolate inter-

mediates acting to modulate photorespiration and serine biosynthesis lend interest to further investigations. Characterization of the putative methanol metabolism and related plant responses may also lead to an understanding of the physiologic impact of endogenous methanol in plant tissue and how leaf-surface methylotrophic bacteria might influence the assimilation of foliar-applied methanol.

At present the physiologic responses to methanol and the pathways of methanol detoxification and assimilation in photosynthesizing plant tissue are poorly understood. The proposed pathways in Fig. 3 suggest that direct assimilation of the carbon from methanol and inhibition of wasteful photorespiration by increased internal CO₂ may be aspects of the mechanisms for putative plant-growth responses (Nonomura and Benson 1992a, 1992b). Interestingly, both of these pathways require rapid methanol oxidation. Yet most alcohol dehydrogenases in plants catalyze negligible levels of methanol oxidation (Eriksson, 1968; Gomes et al., 1982; Grondal et al., 1983; Leblova and Perglerova, 1976; Swaisgood and Pattee, 1968); hence, very little is known about the occurrence of methanol oxidative enzymes in plant tissue. Understanding the pathways of methanol assimilation and detoxification in plants will ultimately require the characterization of the pathway of methanol oxidation in photosynthesizing plant tissue. Alternatively, the work of van Iersal et al. (1995) suggests that the brief enrichment of internal CO₂ concentrations by methanol assimilation is unlikely to be a significant promoter of plant growth. Rather, the longevity and extent of the reported responses in methanol-treated plants suggests the occurrence of some other semipermanent change in C assimilation.

Finally, several authors have offered other explanations of the plant-growth responses to methanol that do not involve metabolism of methanol as a carbon source. Mauney and Gerik (1994) hypothesized that the few observed increases in cotton yield were due to correction of nutrient deficiencies in cotton. Nonomura et al. (1995) found that the addition of iron and nitrogen to methanol solutions eliminated the nutrient-deficiency symptoms that followed repeated applications of methanol to citrus or rice (*Oryza sativa* L.). McGiffen et al. (1995) and Nonomura et al. (1995) noted that methanol may be an effective carrier of many agrichemicals with low water solubility. Mauney and Gerik (1994) and Nonomura and Benson (1992b) observed a decline in pest populations following applications of methanol. The extent of these effects on plant growth remains to be determined.

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