

EFFECTS OF DIFFERENT ETHANOL CONCENTRATIONS ON THE INITIAL GROWTH OF LETTUCE (*Lactuca sativa*)

J. P. Morales-Payan and B. M. Santos. Dominican HortResearch Group, Calle 7 No. 4, Apt. 301, Ens. Julieta, Santo Domingo, Dominican Republic.

ABSTRACT. Greenhouse trials were carried out to determine the effect of different ethanol concentrations on the initial growth of 'South Bay' lettuce. Lettuce plants in the two-true leaf stage were dipped in for 2 minutes in a solution containing 0, 5, 10, 15 or 20% ethanol (v/v). Lettuce fresh weight and plant diameter were collected 30 days after treatment. Lettuce plants that received 10 or 15% dipping had the greatest plant diameter and dry weight compared to other treatments, representing about 21.2 and 19.5% increases in fresh weight, respectively. A 22.2% reduction in fresh weight was observed when plants were treated with 20% ethanol solutions. A tertiary relationship characterized the fresh weight response to ethanol rates ($y = 109.64 - 0.56x + 0.62x^2 - 0.033x^3$; $r^2=0.99$).

INTRODUCTION

Ethanol and methanol have been reported to stimulate seed germination (Adkins et al. 1984; Ho et al. 1995; Smits et al. 1995) and biomass accumulation (Rowe et al. 1994) in a number of plant species. The metabolism of short-chain alcohols in plants and their mechanism of action as plant growth regulators have not been studied in detail and thus are not well understood. Most of the research on this topic has been conducted on methanol. As shown in figure 1, in the proposed methanol pathway in plants, this alcohol undergoes linear oxidation to CO₂ via formaldehyde formation, or cyclic assimilation into carbohydrates, organic acids and amino acids, especially serine and methionine. A similar pathway is thought to occur for ethanol (Cossins 1964; McGiffen and Manthey, 1996).

No growth stimulation due to methanol or ethanol treatment has been reported in C₄ plants such as barley (*Hordeum vulgare*) or maize (*Zea mays*) (Devlin 1994), whereas alcohol applications to Kentucky bluegrass (*Poa pratensis*) were phytotoxic (Crowe et al 1994).

Nonomura and Benson (1992a, 1992b) attributed the different response of C₃ and C₄ plants to treatment with these alcohols to decreased photorespiration rates, stimulation of serine biosynthesis, increased CO₂ availability inside the leaf, and presumed transient modifications in the carbon assimilation processes in C₃ plants. Ethanol and methanol also appear to improve the efficiency of water use in C₃ plants, specially under water stress situations (McGiffen and Manthey, 1996).

In tomato (*Lycopersicon esculentum*), foliar sprays up to 20% ethanol significantly increased shoot fresh and dry weight (Rowe et al.1994). Submersion of bell pepper (*Capsicum annuum*) seedlings in aqueous ethanol solutions resulted in increased plant biomass (Morales-Payan and Santos, 1997). Nonomura and Benson (1992a, 1992b) reported that methanol treatment resulted in biomass increases of 36% in watermelon (*Citrullus lanatus*), 40% in rose (*Rosa* spp.), 50% in savoy cabbage (*Brassica oleracea* Capitata group), tomato and cotton (*Gossypium hirsutum*), 60% in strawberry (*Fragaria x ananasa*) and eggplant (*Solanum melongena*), 70% in palm (*Washingtonia robusta*), and 100% in wheat (*Triticum aestivum*). Enhanced yields have also been reported for methanol-treated soybeans (*Glycine*

max) (Li et al. 1995), radish (*Raphanus sativus*) and peas (*Pisum sativum*) (Devlin et al. 1994). However, according to other reports, methanol has failed to stimulate crop productivity in cotton (Mauney and Gerik 1994), sour orange (*Citrus aurantium*) (Idso et al 1995), carrots (*Daucus carota*), lemon (*Citrus limon*) (McGiffen et al 1995), melon (*Cucumis melo*) (Hartz et al. 1994) and wheat (Albrecht et al. 1995).

In lettuce, McGiffen and collaborators (1995) reported that treatment with 30% methanol was toxic, and that increasing alcohol rates were correlated with increasing plant injury when the crop was grown under desert conditions. Morales-Payan (1997) found no effects of methanol or ethanol at rates up to 20% in 'Black Seeded Simpson' loose leaf lettuce. In the case of lettuce and other C3 plants, the differences in reported results are apparently due to variations in experimental procedures, alcohol rates, environmental conditions and/or genotype responsiveness. The objective of this study was to determine the effect of different ethanol rates on the biomass accumulation of 'South Bay' crisphead lettuce.

MATERIALS AND METHODS

Greenhouse experiments were carried out during spring and summer 1997 at Gainesville, Florida. Crisphead 'South Bay' lettuce was directly seeded in multi-cell flats (3 cm²) and transplanted to 1.5 L plastic containers when seedlings reached the two-true leaf stage. Prior to transplanting, solutions containing 0, 5, 10, 15 or 20% ethanol (v/v) were prepared and seedlings were dipped for 2 minutes into corresponding treatments. A potting medium containing 50% vermiculite, 30% perlite and 20% sphagnum peat (v/v) was utilized and plant nutrients were supplied as recommended by current fertilization practices (Maynard et al. 1995).

Lettuce fresh weight and plant diameter were collected 30 days after treatment (DAT). Data collected were submitted to analysis of variance (ANOVA) and regression analysis was used to assess relationship between fresh weights with ethanol rates. If significant differences ($P \leq 0.05$) were found for fresh weight and plant diameter, treatment means were separated by a Fisher's protected LSD at the 5% significance level.

RESULTS AND DISCUSSION

Ethanol concentrations significantly affected lettuce fresh weight and plant diameter. Maximum fresh weights and plant diameters were measured at 10 and 15% ethanol concentrations representing increases of 21.2 and 25.9%, and 19.5% and 33.3% for fresh weight and diameter at 10 and 15% ethanol, respectively (Figures 2, 3 and 4). A cubic response model described the effect of ethanol concentrations on lettuce fresh weight ($y = 109.64 - 0.56x + 0.62x^2 - 0.033x^3$; $r^2 = 0.99$) and plant diameter ($y = 17.68 - 0.51x + 0.18x^2 - 0.008x^3$; $r^2 = 0.99$). When the concentration of 20% ethanol was used, phytotoxic effects were observed, reducing lettuce fresh weight and plant diameter in 22.2 and 18.5% as compared to the ethanol-free plants. It has been postulated that high rates of alcohols (in this case >20%) are phytotoxic because they overwhelm the enzymatic system that catalyzes ethanol and methanol into other non-toxic carbon compounds. The response trends found in this study appear to agree with the findings of McGiffen et al. (1995), according to which treatments of 30% methanol were toxic to lettuce, but differ from the results reported by

Morales-Payan (1997), who did not detect significant effects of either methanol or ethanol (rates up to 20%) applications to loose leaf lettuce. Differences between the results of the present study and those of McGiffen et al. (1995) and Morales-Payan (1997) could be attributed to cultivar, environmental and procedural variations. Even though the literature presents controversial results about alcohols as plant growth regulators, according to the results found in this study ethanol treatment at rates between 5 and 15% could be beneficial to 'South Bay' lettuce growth and yield.

REFERENCES

- Adkins, S. W., G. M. Simpson and J. Naylor. 1984. The physiological basis of seed dormancy in *Avena fatua*: 6. Respiration and the stimulation of germination by ethanol. *Phys. Plant.* 62(3):148-152.
- Albrecht, S. L., C. L. Douglas, E. L. Klepper, P. E. Rasmussen, R. W. Rickman, R. W. Smiley, D. E. Wilkins and D. J. Wysocki. 1995. Effects of foliar methanol applications on crop yield. *Crop Sci.* 35:1642-1646.
- Cossins, E. A. 1964. The utilization of carbon-14 compounds by plants. 1. The metabolism of methanol-¹⁴C and its role in amino acid biosynthesis. *Can. J. Biochem.* 42:1793-1802.
- Cossins, E. A. 1978. Ethanol metabolism in plants. In: D. D. Hook and R. M. M. Crawford (eds.). *Plant life in anaerobic environment.* Ann Arbor Sci. Publ., Ann Arbor, MI. pp. 169-202.
- Crowe, F. D., D. D. Coats and M. D. Butler. 1994. Performance of Kentucky bluegrass seed treated with methanol. *Central Oregon Agr. Res. Ctr. Annu. Rpt. 1993.* Oregon St. Univ. Special Rpt. 930. pp. 115-120.
- Devlin, R. M. 1994. Influence of methanol on plant germination and growth. *Plant Growth Soc. Amer. Quart.* 22:102-108.
- Hartz, T. K., K. S. Mayberry, M. E. McGiffen, Jr., M. LeStrange, G. Miyao and A. Baameur. 1994. Foliar methanol application ineffective in tomato and melon. *HortScience* 22:1087.
- Ho, C. K., G. Jacobs and D. G. M. Donald. 1995. Effects of sodium hypochlorite, ethanol and culture medium on seed germination of *Paulownia* species. *Seed Sci. Technol.* 23 (1): 157-163.
- Idso, S. B., K. E. Idso, R. L. Garcia, B. A. Kimball and J. K. Hooper. 1995. Effects of atmospheric CO₂ enrichment and foliar methanol application on net photosynthesis of sour orange tree (*Citrus aurantium*; Rutaceae) leaves. *Amer. J. Bot.* 82:26-30.
- Li, Y., G. Gupta, J. M. Joshi and A. K. Siyumbano. 1995. Effect of methanol on soybean photosynthesis and chlorophyll. *J. plant Nutr.* 18:1875-1880.
- Mauney, J. R. and T. J. Gerik. 1994. Evaluating methanol usage in cotton. In: D. J. Herber and D. A. Richter (eds.) *Proc. 1994 Beltwide Cotton Conf., San Diego, CA, vol 1.* Natl. Cotton Council of Amer., Memphis, TN. pp. 39-40.
- Maynard, D., G. Hochmuth, C. Vavrina, W. Stall, T. Kucharek, F. Johnson and T. Taylor. Lettuce, endive and escarole production in Florida. In: D. Maynard and G. Hochmuth (eds.) *Vegetable Production Guide for Florida.* Univ. of Florida SP-170. pp. 185-196.
- McGiffen, M. E., R. L. Green, J. A. Manthey, B. A. Faber, A. J. Downer, N. J. Sakovich and J. Aguiar. 1995. Field tests of methanol as crop yield enhancer. *HortScience* 30:1225-

- McGiffen, M. E. and J. A. Manthey. 1996. The role of methanol in promoting plant growth: a current evaluation. *HortScience* 31(7): 1092-1096.
- Morales-Payan, J. P. 1997. Influence of methanol, ethanol and nitrogen on the yield of lettuce (*Lactuca sativa*). *HortScience* 32(3):437.
- Morales-Payan, J. P. and B. M. Santos. 1997. Initial Bell pepper (*Capsicum annuum*) growth as influenced by different leaf-applied ethanol concentrations. 1997 Proc. Carib. Food. Crops Soc. San Juan, PR.
- Nonomura, A. M. and A. A. Benson. 1992. The path of carbon in photosynthesis: Improved crop yields with methanol. *Proc. Natl. Acad. Sci. USA* 89 (20): 9794-9798.
- Rowe, R. N., D. J. Farr. and B. A. J. Richards. 1994. Effects of foliar and root applications of methanol and ethanol on the growth of tomato (*Lycopersicon esculentum* Mill). *New Zealand J. Crop Hort. Sci.* 22 (3): 335-337.
- Smits, A. J. M., G. H. W. Schmitz, G. Van Der Velde and L. A. Voesenek. 1995. Influence of ethanol and ethylene on the seed germination of three nymphaeid water plants. *Freshwater Biol.* 34 (1): 39-46.

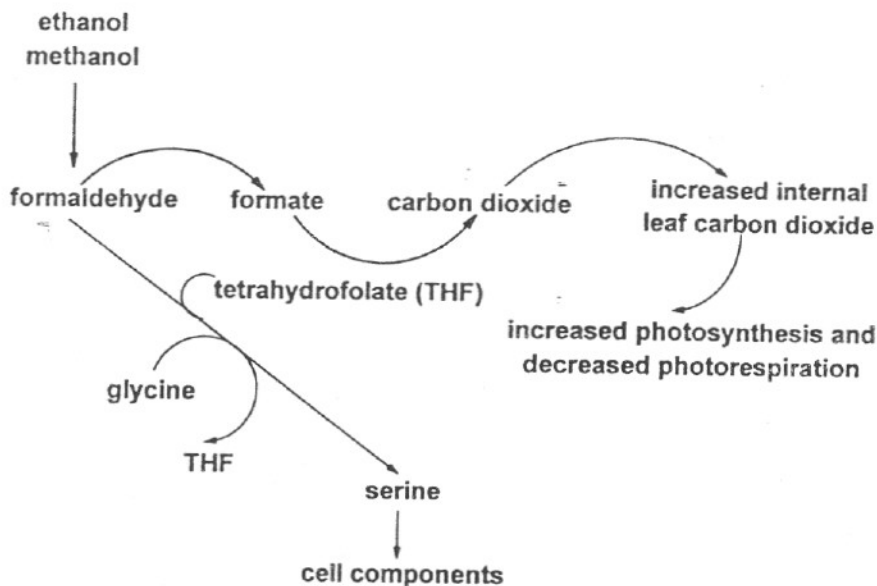


Figure 1. Proposed mechanisms of growth enhancement of methanol-ethanol treated C3 plants (Modified from McGriffen and Manthey, 1996).

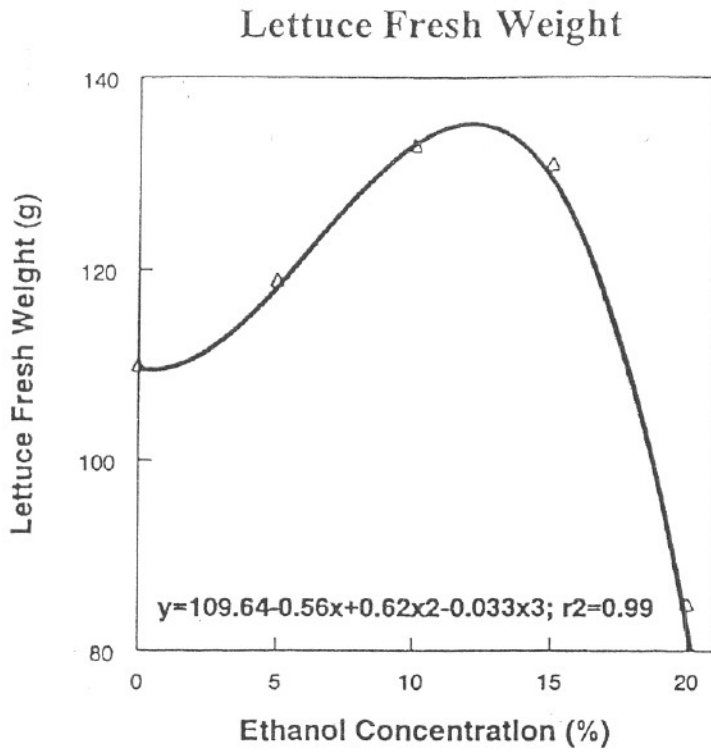


Figure 2. Lettuce fresh weights per plant as affected by ethanol concentrations.

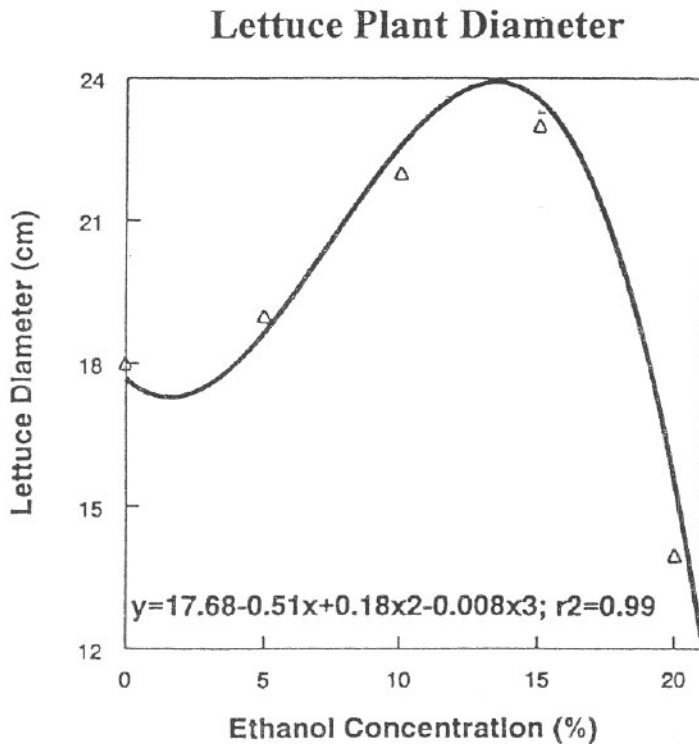


Figure 3. Lettuce plant diameter as affected by ethanol concentrations.

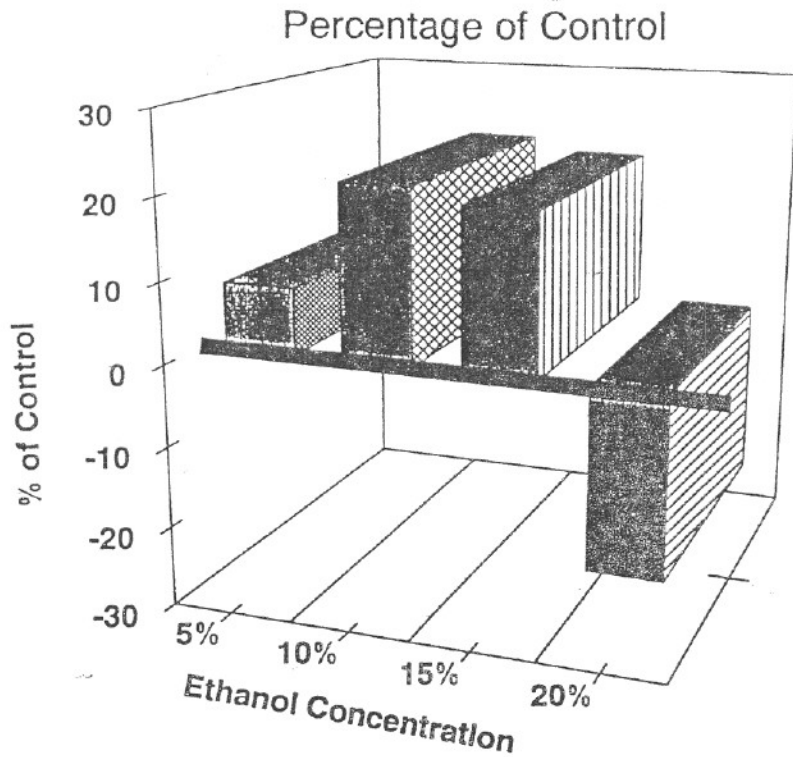


Figure 4. Lettuce fresh weights as percentage of ethanol-free control.