Phytotoxicity of Steam Additive Diethylaminoethanol to Chrysanthemums

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Chlorosis in leaf tissue of plants may result from a variety of causes: these include iron and other mineral deficiencies, excess soluble salts and root injury, photoperiod-high light intensity or even viroid infections. Recently it was established that certain plants grown in steam-sterilized soil or in an atmosphere in which relative humidity was controlled by steam-generated mist also exhibited chlorosis. The active ingredient in the steam was found to be 2-diethylaminoethanol (DAE). DAE, a neutralizing amine, is added to steam generators to prevent acid corrosion of steam condensate lines. It is a stable and highly water soluble compound.

The DAE used in these studies was that sold commercially as Pennad 150 (99.5% purity) as well as commercially pure DAE (99 + % purity) from Aldrich Chemical Company. DAE was introduced into plants by three methods. 1) Plants grown in nonsterile soil to which DAE was added in a single application of 10-fold aqueous concentrations from 1 to 0.00001 ml DAE/5 inch pot. 2) Soil in which plants were grown was autoclaved with DAE-containing steam. 3) Plants were grown in controlled environment chambers in either nonsterile or DAE-steam-sterilized soil and in which atmospheric humidity was enhanced by DAE-containing steam.

Several cultivars of *Chrysanthemum morifolium* were used in these investigations: 'Bonnie Jean', 'Velvet Ridge' and 'Indianapolis White' obtained from The California-Florida Plant Corp., Fremont, CA and 'Mistletoe' and 'Blanche' from my own laboratory at Cornell University.

Plants were grown individually in potting soil consisting of 1 part peat moss : 2 parts vermiculite and amended with lime. A fertilizer solution (6 g 0-20-0 + 6 g 10-10-10/1 water) was applied at each watering at the rate of 15:1 (water:fertilizer). All plants were grown in 5 inch diameter plastic pots. Plastic saucers were placed under all pots during the experimental period.

Greenhouse experiments were performed at 28±2°C with a minimum light intensity of 10,700 lux (incident light and cool white fluorescent) and 16 hr photoperiod. Additional experiments were carried out in controlled environment chambers.

Chrysanthemums 'Bonnie Jean' and 'Velvet Ridge' were inoculated with a symptomless strain (ChCMV-L) of chrysanthemum chlorotic mottle viroid (ChCMV) by tissue implantation. The protection of ChCMV-L inoculated plants against subsequent challenge inoculations with a severe strain of ChCMV verified the presence of ChCMV-L infection; by this challenge test, 'Mistletoe' and 'Blanche' were found to have been previously infected with ChCMV-L.

Foliar concentrations of N, P, Ca, K, Mg, Fe, Na, Zn, Mn, Cu, B, and Al were determined in 'Indianapolis White' chrysanthemums which had been exposed to 0, 0.0001, and 0.1 ml DAE added to nonsterile soil. In addition, P and K levels in leaves of uninfected 'Velvet Ridge' and uninfected and ChCMV-L infected 'Bonnie Jean' exposed to 0, 0.001, 0.01, 0.1 or 1 ml DAE/pot were assayed. All plants used for nutrient analysis were grown at 28°C, 16-hr photoperiod for 60 days following DAE treatment. Two replicates of four plants were tested for each treatment; the 5th, 6th, and 7th youngest and expanded leaves of each plant were selected for analysis. Analyses were performed by the Tissue Testing Laboratory, Department of Floriculture and Ornamental Floriculture, Cornell University. Statistical analysis was performed on the data using the Duncan's Multiple Range Test.

DAE was found to cause chlorosis in young leaves of chrysanthemums. Chrysanthemum cultivars varied in their sensitivity to DAE. Symptoms were generally restricted to leaves which developed after initiation of DAE exposure, and included chlorosis, epinasty, stunting, and necrosis (Figure 1). Chrysanthemum cultivar Indianapolis White was sensitive to low concentrations of DAE, while 'Bonnie Jean', 'Velvet Ridge', and 'Mistletoe' were less so. All 'Indianapolis White' exhibited chlorosis 7 days after exposure to 0.01 ml DAE added to nonsterile soil or after 11 days at 0.001 ml added DAE; at 0.0001 ml, only 50% of the plants were chlorotic after 22 days. Healthy 'Bonnie Jean' and 'Velvet Ridge' did not exhibit an effect from DAE until applied at levels of 1 ml/pot and 0.1 ml/pot, respectively (Table 1). When subjected to DAE either separately or in combination in the atmospheric humidity or in steam-sterilized soil, all 'Indianapolis White' became chlorotic regardless of growth temperature (24 or 30 C), while 'Mistletoe' did not exhibit chlorosis unless DAE was present in both forms (Table 2). In the absence of DAE, under these conditions, 'Bonnie Jean' and 'Mistletoe' remained symptomless.

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Phototoxicity (continued)

The effect of DAE on healthy and ChCMV-L infected chrysanthemums was compared in 'Bonnie Jean' and 'Velvet Ridge' (Table 1). The presence of ChCMV-L increased the sensitivity of 'Bonnie Jean' and 'Velvet Ridge' to DAE. Viroid-infected plants of both cultivars exhibited DAE sensitivity at a 100-fold lower level than that producing symptoms in healthy plants. 'Blanche' and 'Mistletoe', both naturally-infected with ChCMV-L, were found to be sensitive to DAE; however, since no ChCMV-L free plants of these cultivars could be obtained, it was not possible to assess the effects of the viroid on DAE sensitivity in these chrysanthemums.

Leaves of chrysanthemum exhibiting DAE-induced chlorosis contained higher but non-toxic levels of Fe and other minerals than those of non-DAE-treated plants. Nutrient analysis of leaves of DAE-treated 'Indianapolis White' indicated higher levels of N, P, Ca, K, Fe, Na, Mn, and B than those present in leaves of untreated plants. DAE did not affect the levels of Mg, Zn, Cu, or Al. Additional studies of P and K content in uninfected 'Velvet Ridge' and in uninfected and ChCMV-L infected 'Bonnie Jean' which were exposed to various levels of DAE indicated that the amounts of both nutrients increased at higher DAE levels (0.1, 1.0 ml/pot) regardless of the presence of viroid infection. This increase was associated with DAE-induced chlorosis (Table 1).

Other neutralizing amines such as morpholine and cyclohexamine have been reported by other workers to be phytotoxic to plants as well (reports by Drummond and Vasiloff; Huges and Sangster; Koths and Judd). Koths and Judd in 1976 reported that chrysanthemum cultivars 'Southern Sun', 'Nimbus', 'Symphony' and 'Bonnie Jean' showed a variety of phytotoxic symptoms to Mogul 7300 (10% DAE) and that symptom severity was cultivar-dependent. The findings at Cornell University corroborate these earlier reports. Furthermore viroid infection increased the level of DAE-induced chlorosis in chrysanthemums.

Unlike viroid-induced chlorosis, DAE-induced symptoms are not temperature or photoperiod-dependent. The higher levels of DAE induced elevated levels of some foliar minerals as well; however, these levels are not in the range of toxicity (J. W. Boodley, personal communication).

The presence of DAE in steam presents many types of problems. Foremost is the chlorosis-type of phytotoxicity. DAE can interfere with nutritional studies by altering the concentration of many elements in the leaves. Its presence in steam-sterilized soil may also lead to ineffective fertilization practices; in spite of the abundance of Fe in DAE-affected leaves, moderate levels of DAE causes interveinal chlorosis identical in appearance to that caused by Fe deficiency.

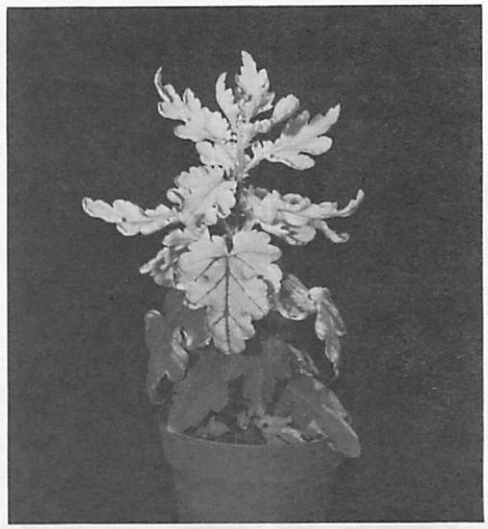

Figure 1. DAE-induced chlorosis in chrysanthemum cultivar 'Indianapolis White'.

Methods to avoid DAE-toxicity of plants might include the use of 1) materials other than neutralizing amines to prevent corrosion of steam condensate lines, 2) electric autoclaves or steam generators with corrosion-resistant pipes, 3) nonsterile soil, 4) long-term storage of steam sterilized soil prior to use, or 5) resistant or non-sensitive plants.

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TABLE 1. Effect of DAE added to the soil of healthy and ChCMV-L infected chrysanthemums (22-day exposure; 16-hr photoperiod).

ml DAE /pot	Cultivar: Indianapolis White	Bonnie Jean: Healthy	Bonnie Jean: ChCMV-L	Velvet Ridge: Healthy	Velvet Ridge: ChCMV-L
0	0/4†	0/8	0/8	0/5	0/5
10^{-4}	2/4	—‡	—	0/5	0/5
10^{-3}	4/4	0/8	0/8	0/5	2/5
10^{-2}	4/4	0/8	1/8	0/5	2/5
10^{-1}	4/4	0/8	8/8	2/5	3/4
10^{0}	4/4	8/8	8/8	5/5	5/5

† number of chlorotic/number of experimental plants
‡ not tested

TABLE 2. Effect of DAE in steam-treated soil and in atmospheric humidity on two cultivars of chrysanthemum (24-day exposure; 30 C; 16-hr photoperiod).

Treatment	Cultivar: Indianapolis White	Cultivar: Mistletoe
Unsterilized soil		
DAE-containing humidity	5/5†	0/6
DAE-free humidity	1/5	0/6
Soil autoclaved with DAE-treated steam		
DAE-containing humidity	4/4	1/6
DAE-free humidity	3/4	0/6

† number of chlorotic/number of experimental plants