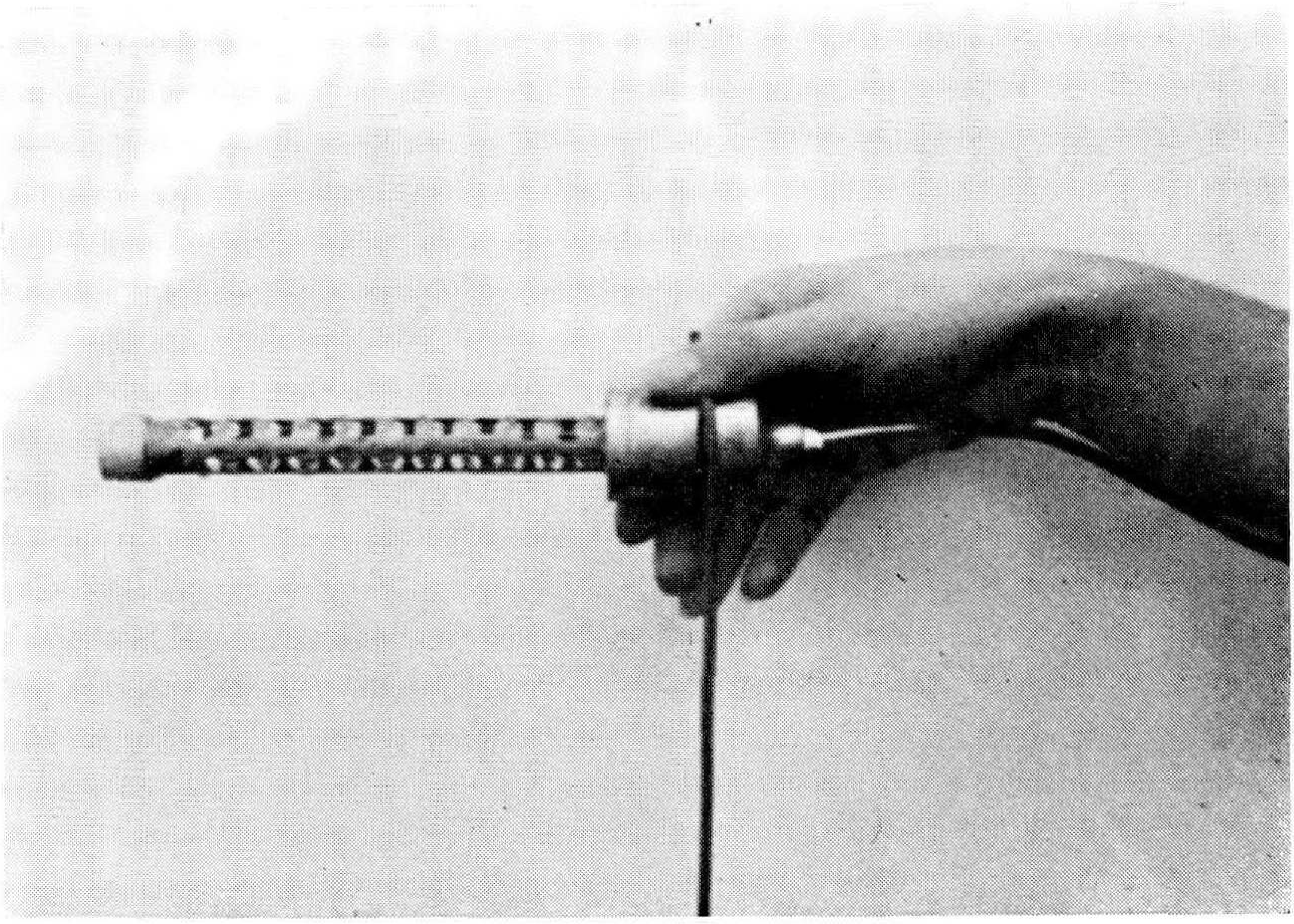

Figure 3. A finished model of the aqua-vapor control.

STU NELSON: Our next subject is one which stimulated much discussion last year — DMSO. To lead off the discussion this afternoon is Dr. Len Stoltz.

EFFECT OF DIMETHYLSULFOXIDE(DMSO) AND TOBACCO SMOKE EXTRACT (TSE) ON ROOT INITIATION

LEONARD P. STOLTZ
Department of Horticulture
University of Kentucky
Lexington, Kentucky

While working in Dr. Hess' lab I discovered that tobacco smoke blown into a piece of filter paper had a strong root initiating effect on mung beans which are used in the rooting bioassay developed by Hess (1). Later tobacco smoke extract which had been prepared by collecting the smoke in a cold trap from machines which automatically smoke the cigarettes was purchased and used. On the average 50 cigarettes yields 1 gram of tar.

In order to obtain an indication of the strength of the root initiation effect one gram of TSE was dissolved in 10 ml of methyl alcohol. A serial dilution was prapared by reducing to one-half the amount of TSE for seven dilutions; the last dilution contained 1/128 as much as the first. Twenty lambda of each extract was spotted on filter paper and tested using Hess' mung bean rooting bioassay. In this case 1 on the ordinate represents 0.05 cigarette or 2 mgr. of TSE. Maximum activity of 68 roots per cutting was obtained with 1 mgr of TSE.

When TSE is chromatographed in different solvents the active fraction moves to the same area of the chromatogram as does Hess' cofactor 4. Because of this and its physiological activities the two are considered to be the same or very similar in chemical make up.

TSE also promotes the growth of oat mesocotyl sections using the methods of Nitsch and Nitsch (2). The material which promotes oat mesocotyl section occurs at Rf 0.4 to 0.5 while the fraction which promotes root initiation occurs at Rf 0.8 to 0.95.

Because of the striking root and growth promotive effects of TSE I was curious as to whether this material might be of value in the propagation of difficult-to-root cuttings. Preliminary tests using TSE alone showed no striking effect on the rooting of coleus, chryanthemum or hibiscus.

Dimethylsulfoxide (DMSO) is reportedly effective in aiding the movement of various materials across plant and especially animal tissues. The failure of TSE in the preliminary tests to influence rooting could have been the result of its inability to cross membranes and move to its site of action. Therefore DMSO alone and in combination with auxins and TSE were tested on the rooting of mung bean particularly a difficult-to-root Chrysanthemum variety Mrs. Roy.[1] DMSO at

[1]The author expresses his gratitude to Yoder Bros, Inc, Barberton, Ohio who furnished the the Chrysanthemum cuttings, varieties Mrs Roy and Bright Golden Anne used in these studies

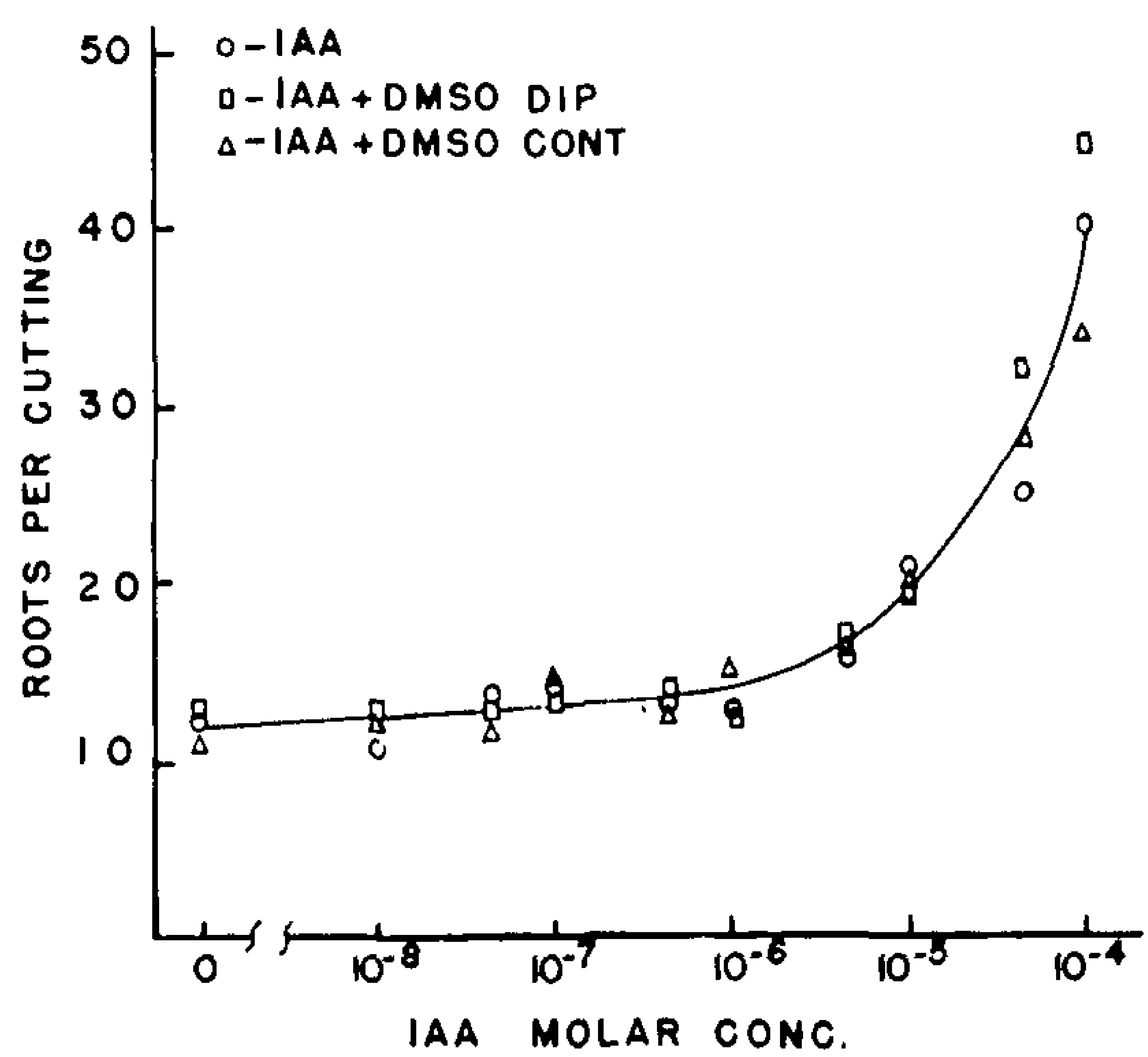

Figure 1 Root initiation of mung beans when treated with several concentrations of IAA. O-IAA supplied alone, O-IAA plus, 5% DMSO as a dip treatment and O-IAA and 50 ppm DMSO supplied continuously to the cuttings.

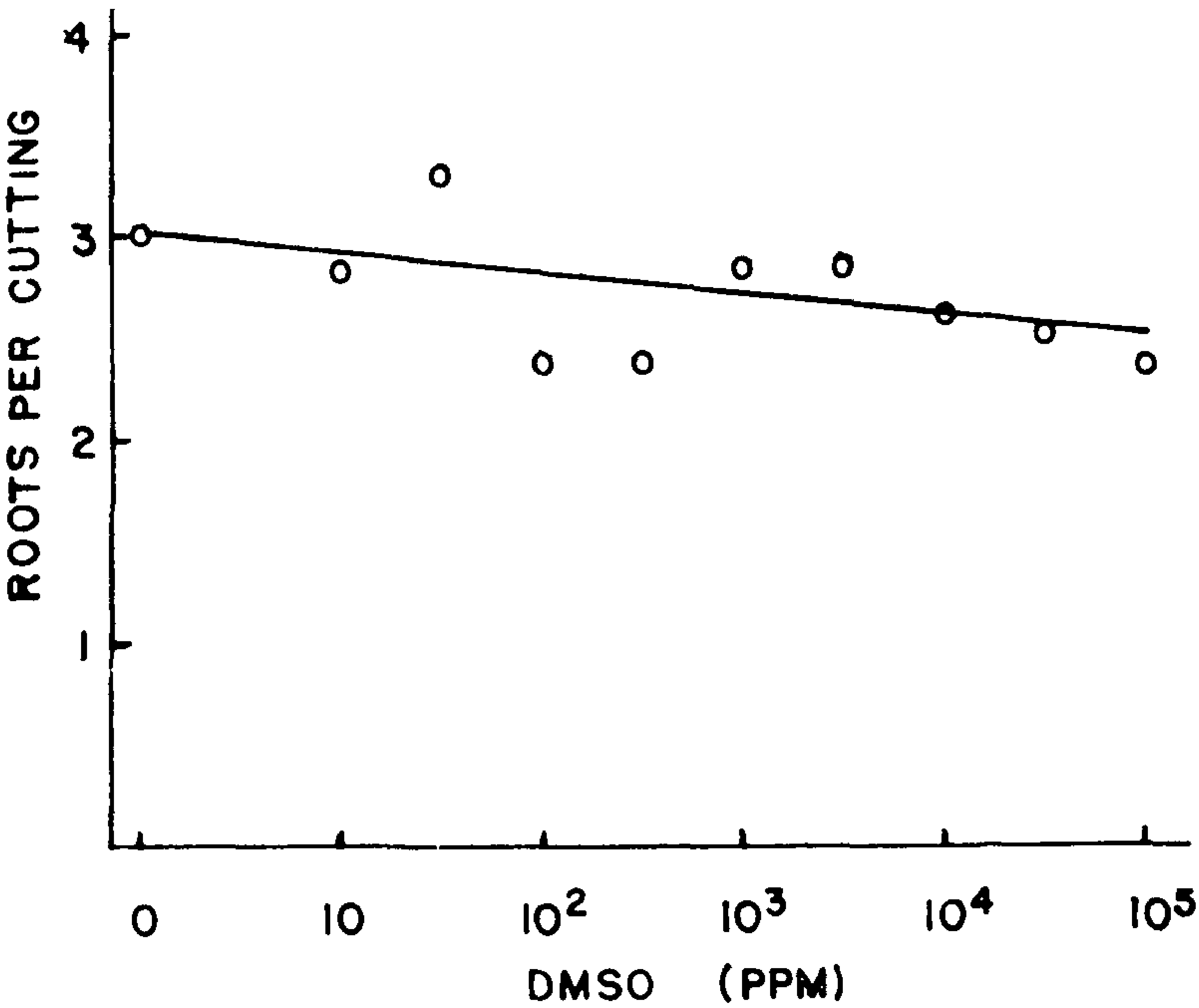

Figure 2 Root initiation response of Chrysanthemum variety Mrs. Roy as a result of dipping the basal ends of the cuttings into several concentrations of DMSO

1000 ppm and above was toxic when supplied continuously to mung beans but no deleterious effects were observed up to 100,000 ppm (10%) when the cuttings were dipped into it and transferred to vials of water for rooting.

Indoleacetic acid with and without DMSO was first tested on mung bean seedlings to determine if the DMSO promoted the effects of IAA. No promotive effect was found. The results are presented graphically in Figure 1.

In the next series of tests only the difficult-to-root Chrysanthemum variety Mrs. Roy was used. In the first test the base of the cuttings were dipped in DMSO and the cuttings were stuck in a mist propagation bed for rooting. DMSO at 10, 10^2, 10^3, 10^4 and 10^5 ppm was used. There was a slight depression of roots per cutting as the concentration of DMSO increased. See Figure 2.

The auxin and rooting cofactor activities of extracts of the basal portions of the Chrysanthemum cuttings used in the next 3 tests were determined. The auxin activity was about the same for each group of cuttings of Mrs. Roy used and showed little difference from the easy-to-root variety, Bright Golden Anne, which was assayed to determine if auxin content alone could account for the reduced rooting response of Mrs. Roy.

The second test was undertaken to determine if 10% DMSO used as a carrier for indolebutyric acid (IBA) would increase the rooting response of Mrs. Roy cuttings. Concentrations of 100, 500, 1000 and 2000 ppm IBA were used. The number of roots per cutting increased as the concentration of IBA increased but DMSO had no enhancing effect and is considered to have had a slight inhibitory effect. See Figure 3. The rooting cofactor activity of the basal stem portion of Mrs. Roy cuttings was low compared to that in the easy-to-root variety but the leaves of Mrs. Roy showed greater amounts of cofactor 4 than the leaves of Bright Golden Anne. Thus in the stem area where rooting occurs there is a lesser concentration of rooting cofactor 4.

In the third test the basal ½″ of cuttings of Mrs. Roy were dipped in 50% alcoholic solution solutions of TSE with and without 10% DMSO. Some increase in rooting occurred as the concentration of TSE increased but this amounted to on-

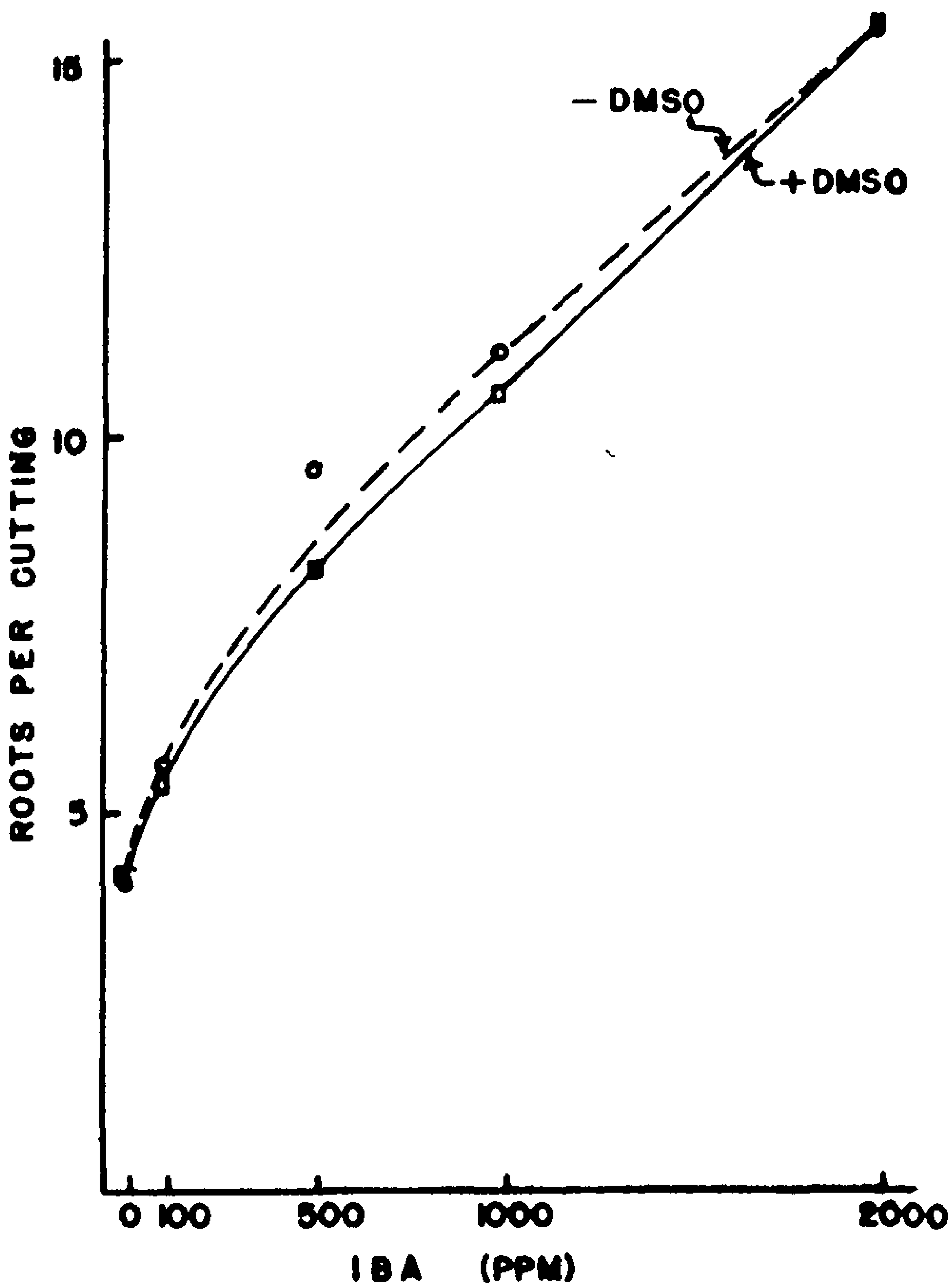

Figure 3. Root initiation of Chrysanthemum variety Mrs. Roy when treated with several concentrations of IBA alone and IBA plus 10% DMSO.

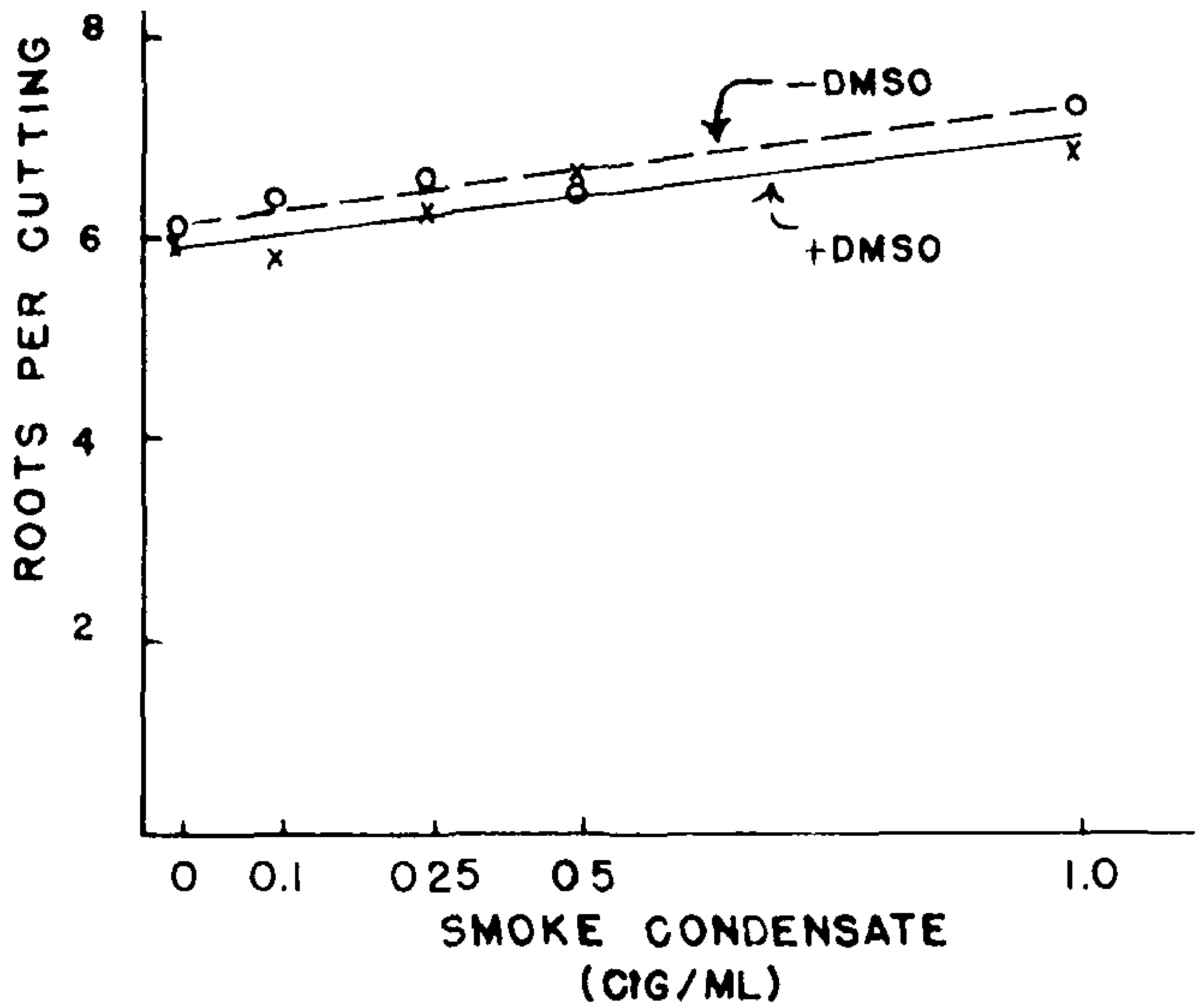

Figure 4 Root initiation of Chrysanthemum variety Mrs Roy when treated with several concentrations of tobacco smoke condensate alone and with 10% DMSO

ly one root per cutting at the highest concentration used. See Figure 4. DMSO appeared to be slightly inhibitory. The cofactor 4 concentration in the stem tissue of Mrs. Roy cuttings was only slightly less than that in the Bright Golden Anne. As previously noted the auxin contents of these two varieties were also about equal in each test. In this case the Mrs. Roy and Bright Golden Anne each had about the same relative amounts of cofactors and auxin yet Bright Golden Anne had an average of 27 roots per cutting while Mrs. Roy had only 7 roots per cutting. This indicates that some factor other than auxin and cofactors is limiting in the cuttings of Mrs. Roy. This became more clear when the results of the fourth test were completed. The rooting cofactor activity of the basal stems of this group of Mrs. Roy cuttings was the lowest of all. Therefore one would assume that if cofactor 4 was the limiting factor these cuttings would exhibit the lowest rooting and be more likely to respond to applied cofactor 4.

In the fourth test the cuttings were allowed to take up TSE with and without 1000 ppm DMSO for 14 hours before being stuck in the mist bed. No promotion by TSE with or without DMSO occurred and the higher concentrations used gave reduced rooting. The interesting fact noted in this case was that rather than having the lowest rooting response, as might be predicted from the cofactor and auxin bioassays, the untreated cuttings in this group averaged 13 roots per cutting. The mum rooting tests were run in March, April, May and

June respectively. Since daylength was increasing during this time it is assumed that carbohydrate content would also be increasing. Stoltz and Hess (3,4) have shown a direct relationship between both cofactor 4 and carbohydrates in the rooting of girdled Hibiscus cuttings. It is proposed that in the tests reported here the rooting of Mrs. Roy cuttings was related to the carbohydrate content more so than to the cofactor or auxin content. However the number of roots per cutting of Mrs. Roy is still considerably less than that of the easy-to-root variety Bright Golden Anne and some factor other than carbohydrates, auxin and rooting cofactors is involved in its reduced root initiation response.

The conclusions drawn from the tests reported here are as follows:

1. DMSO causes a slight inhibition of root initiation in cuttings of mung bean and Chrysanthemum variety Mrs. Roy.
2. TSE causes pronounced root initiation in cuttings of mung bean but fails to give any response when applied to cuttings of Chrysanthemum variety Mrs. Roy.
3. Some factor(s) other than auxin, rooting cofactors and carbohydrates appear to be responsible for the reduced root initiation in Chrysanthemum variety Mrs. Roy.

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HUGH STEAVENSON: Thank you very much for that very interesting research report. Our next speaker is Dr. Booker Whatley.