asci produced by the unirradiated diploid cells contained 4 viable spores. Asci arising from the irradiated diploid cells, however, showed a statistically significant reduction in the percentage of viable spores which they contained (P < 0.001). The majority of the asci having reduced percentage germination contained either 2/4 or 4/4 inviable spores. This is indicative of segregation of lethal gene or chromosomal mutations. These mutations most likely are recessive since, at the doses used, the diploid cell that carried them would still have been viable for vegetative reproduction.

Although this experiment demonstrates the presence of x-ray induced recessive lethal mutations in yeast, it still does not prove that this is the only form of lethal damage induced. Extension of the radiobiological data on haploid and diploid yeast to triploid and tetraploid yeast cells (6, 7) indicates that some other form of lethal damage is also induced by the x-rays. This damage has been proposed to be in the form of dominant lethal mutations.

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## Secondary Galls and Other Plant Growth-Modifying Effects Induced by Translocated a-Methoxyphenylacetic Acid

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Several well-known growth-modifying compounds that induce cell proliferation (gall formation) such as 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, and naphthoxyacetic acid are composed of a ring nucleus and the acetic acid side chain, these two parts being associated through oxygen thus forming an ether linkage (1, 2). Alpha-methoxyphenylacetic acid (MOPA), prepared by Reeve and Christoffel (3), has an ether linkage associated with a carbon of the acetic acid side chain rather than with a carbon of the ring nucleus. In the present investigation a-methoxyphenylacetic acid and several related compounds<sup>2</sup> were studied to determine their

effects on cell proliferation and other growth responses.

In preliminary screening tests MOPA greatly modified vegetative growth of cucumber, snap bean, and sunflower plants. It also caused primary cell proliferation (gall formation in the treated portion of the stem) and secondary cell proliferation in the stems of bean plants, the secondary galls being formed some distance from the primary ones. These and other growth responses were therefore studied in detail by applying MOPA to several kinds of crop plants.

A lanolin-Tween 20 mixture of the compound was prepared by dissolving 25 mg of MOPA in 0.5 g of Tween 20 and thoroughly mixing with 2 g of melted lanolin. Approximately 12 mg of the mixture was applied to the stem of each plant by means of a narrow glass rod. Various dosage levels were obtained by diluting aliquots of the original 1% mixture with the required amounts of the lanolin-Tween 20 carrier. The paste was placed as a band approximately 3 mm wide around the stem midway between the first and the second node of bean and between the second and the third node of tomato and on the upper portion of the hypocotyl of sunflower and cucumber plants. Approximately the same amount of paste was applied as a strip about 1 cm wide and extending across the upper surface of the first leaf of barley and corn plants. All plants were grown in a greenhouse and treated at an early stage in their development.

The initial response by tomato and bean plants was a moderate and temporary stem curvature. In addition, primary leaf petioles of bean plants curled temporarily downward, the sides of the leaves folding upward at the midrib and thus exposing the under surfaces.

Later responses of cucumber, sunflower, and bean plants included suppression of terminal growth accompanied by increased growth of axillary shoots. The compound delayed flowering and production of fruit by bean plants. The terminal growth of tomatoes was not suppressed, but development of axillary shoots was stimulated. Axillary shoots of treated tomato plants grew in length 59 times as much as did those of comparable untreated ones. No delay in flowering resulted from application of the chemical. Response of tomato leaves was marked, varying from a reduction in the size of leaflets to a reduction in their number. MOPA had a similar effect on the development of bean leaves and in addition some 4- and 5-lobed leaves developed.

Growth of corn and barley plants was not apparently affected by MOPA applied at various concentrations including 1, 0.5, 0.25, 0.13, 0.06, and 0.03%. In contrast, growth of the bean and tomato plants was affected by MOPA at all these concentrations, the least effect occurring at the lowest dosage level.

So that the growth-modifying effects of MOPA could be compared with those of phenylacetic acid, each compound was applied at 4 concentration levels (1, 0.5, 0.25, and 0.1%) to the first internodes of some bean plants and to the second internodes of

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<sup>&</sup>lt;sup>2</sup> Samples obtained from W. Reeve, University of Maryland, through the Chemical-Biological Coordination Center, Na-tional Research Council.

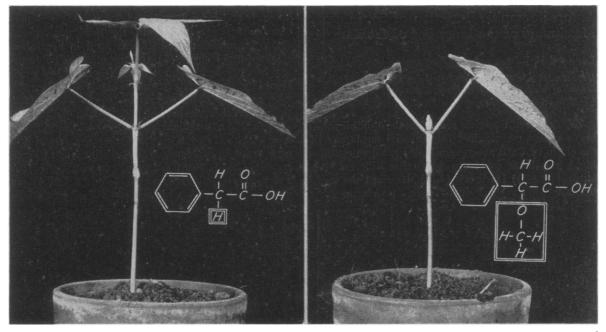


FIG. 1. Response of bean plants to phenylacetic (left) and methoxyphenylacetic acids (difference in structure emphasized by double-line boxes) applied to the first internodes. Both compounds induced primary galls within the treated areas; the latter compound induced an additional gall within the second internode.

others. The average fresh weight of primary galls induced by phenylacetic acid on first internodes and on second internodes was not significantly different from that of those induced by MOPA at any of the dosage levels used; there was an indication that MOPA was somewhat less active at the two lower dosage levels. When MOPA at the 1% level was applied to the first internodes, the secondary gall on each plant resulted from proliferation of cells in the second internode and was accompanied by almost complete inhibition of growth of parts above this internode (Fig. 1).

Apparently sufficient MOPA was translocated from the site of application to the second internode to induce cell proliferation. That MOPA induced bean stems to develop secondary galls is not unique, however, since several compounds related to 2,4-dichlorophenoxyacetic acid have induced similar responses (4). Development of malformed leaves on shoots from axillary buds originating below the stem areas to which MOPA was applied indicated that this growthmodifying substance was also moved in a downward direction through bean stems. Under the conditions described, substitution of the methoxy group for hydrogen (indicated by double line boxes, Fig. 1) on the alpha carbon of phenylacetic acid resulted in a compound that may have been somewhat more readily translocated by the plant than was phenylacetic acid, although both compounds apparently possess about the same primary gall-inducing activity. MOPA, phenylacetic acid, and 9 related compounds were tested on bean plants at 1% concentration to compare their effectiveness in causing cellular proliferation and other growth-modifying effects. Most growth modification resulted from application of MOPA. Substitution of hydrogen for the ring nucleus (methoxyacetic acid) eliminated the gall-forming response, but there was marked anthocyanin development in the second internode. Benzyl methyl ether, a-methoxyacetophenone, and the following compounds having a hydroxyl group on the side chain proved to be inactive: 1-methoxypropanol-2, 2-methoxypropanol-1, 2-phenyl-2-methoxyethanol, 2-methoxy-1phenyl-ethanol-1, and mandelic acid. The sodium acid salt of MOPA (3) caused growth modifications similar to those induced by MOPA.

The plant growth-modifying activity of some compounds such as benzoic and phenoxyacetic acids was greatly increased by substitution of halogens or certain groups of atoms for some of the hydrogen atoms associated with the benzene ring (2). It is apparent in the present experiments that MOPA, which is similar in structure, was relatively active as a growthmodifying compound without substitution in the benzene ring. Importance of chlorine substitutions on the benzene ring of MOPA is now being investigated. The effects of such well-known growth-modifying substances as indoleacetic, naphthoxyacetic, and 2,4dichlorophenoxyacetic acids following substitution of the methoxy group for a hydrogen associated with the alpha carbon may also be of interest.

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