The Effect of Light and Humidity on Absorption and Degradation of Diphenamid in Tomatoes

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The effect of various light and relative humidity regimes on the absorption and degradation of *N,N*-dimethyl-2,2-diphenylacetamide (diphenamid) by tomatoes (*Lycopersicon esculentum* Mill.) was studied by extracting plants which were grown for 24, 48, and 120 hr in a nutrient solution containing ^{14}C-diphenamid. Plants grown under low light, low humidity conditions absorbed more diphenamid than those grown under high light, high humidity. Extracts of plants grown under high light, high humidity conditions contained a lower percentage of diphenamid and a higher concentration of a glucose–diphenamid complex than those grown under low light, low humidity conditions. The concentration of diphenamid increased in the shoots of plants grown under low light, low humidity but remained constant in plants grown under high light, high humidity. The concentration of the glucose–diphenamid complex in shoot extracts was nearly the same at all harvest periods and in all treatments.

Diphenamid (MMDA) is applied preemergence to control a wide variety of weeds in direct-seeded and transplanted tomatoes (Alder *et al.*, 1960). Lemin (1966) reported that the resistance of tomatoes to diphenamid is due to the conversion of the herbicide to the less toxic compound *N*-methyl-2,2-diphenylacetamide (MDA) in the plant. Kesner and Ries (1967) reported that MDA and 2,2-diphenylacetamide (DA) are more inhibitory to tomato seedlings than diphenamid. Gentner (1969), however, stated that the parent compound is more toxic to tomato and three other plant species than MDA, DA, or 2,2-diphenylacetic acid (DAA).

In 1971, Lynch and Sweet reported that tomato seedlings growing in the greenhouse are injured by applications of diphenamid. They also found a highly positive correlation between low light intensity and injury by diphenamid.

This investigation was designed to determine the effect of low light and low relative humidity *vs.* high light and high humidity on the absorption and degradation of diphenamid of tomato plants in order to explain the injury sometimes observed to tomato plants.

MATERIALS AND METHODS

Chemicals and Counting Procedure. The ^{14}C-MMDA used in these experiments was labeled in the carbonyl position and had a specific activity of 10.7 μCi/mg. The following ^{14}C-carbonyl-labeled metabolites were used as standards for chromatographic comparisons: *N*-methyl-2,2-diphenylacetamide (MDA), 2,2-diphenylacetamide (DA), and 2,2-diphenylacetic acid (DAA). Pure nonlabeled diphenamid, MDA, DA, and DAA were also used. All were checked for purity by thin-layer chromatography (tlc) and radioautography and each chemical had a purity of 98% or better. A liquid scintillation counter was used to measure the radioactivity. Scintillation fluids and methods were the same as those previously reported (Schultz and Tweedy, 1971). The amount of quenching was determined by use of an internal standard (^{14}C-toluene) and all radioactive measurements are expressed as DPM. The location of radioactive compounds on thin-layer plates was determined by radioisotope strip scanning and by radioautography.

Growth Chamber Conditions. For the low light regime, one growth chamber was set at 400 ft-candles, 14-hr day, 10-hr night; relative humidity, 44 to 50% day, 75 to 85% night; temperatures, 22 to 24° C day, 13° C night. For the high light regime, a second growth chamber was set at 1500 ft-candles, 14-hr day, 10-hr night; relative humidity, 80 to 88% day, 85 to 95% night; temperature, 22 to 24° C day, 13° C night.

Plant Material and Treatment. Tomato seeds were germinated in sand in a growth chamber. After emergence the plants were watered every 2 days with half-strength Hoagland's nutrient solution. The seedlings were removed when they were 21 days old, rinsed free of sand, and 10 to 12 plants (5 to 7 g total) were placed in 100-ml beakers which contained 50 ml of sterile half-strength Hoagland's solution. One microcurie of ^{14}C-diphenamid was added to the solution in each beaker and half of the beakers were placed in each of the two growth chambers for 24, 48, or 120 hr. At the end of each of these periods, one-third of the plants were removed from each growth chamber. The roots were thoroughly rinsed with distilled water and blotted dry with absorbent paper. The roots were excised from the shoots and both were weighed and either homogenized or frozen immediately.

A similar study utilizing excised shoots of tomatoes was conducted simultaneously to simulate foliar applications of the herbicide. The procedures were identical to those described above except that the shoots were excised under water. The base of the shoots was kept under solution throughout the remainder of the experiment. All experiments were repeated three times with duplicate treatments in each experiment.

Tissue Extraction. The plant tissue was homogenized in

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a Virtis "45" homogenizer for 2 min in 30 ml of anhydrous methanol. The homogenate was filtered under vacuum through Whatman No. 1 filter paper and the residue was thoroughly washed with methanol:acetone (1:1 v/v) until free of pigment. The filtrate and wash were combined, the volume was measured, and the radioactivity was determined. The combined filtrate–wash was prepared for tlc by forcing it through a column (15 × 2.3 cm) of 10 ml each of anhydrous sodium sulfate, animal charcoal (Fisher Scientific Co., cat. no. 263), and nonactivated florisil from top to bottom, respectively. The florisil was used as received and was purchased from Fisher Scientific Co. (cat. no. F-100, 60–100 mesh). Before use the column was washed with 20 ml of methanol. Recovery of radioactivity from the column was between 95 and 98% for each experiment. The eluent and wash from the column were combined and evaporated under a stream of dry air. The residue was dissolved in 2 ml of methanol and this solution was spotted on tlc plates.

Separation, Detection, and Identification of Diphenamid and Metabolites. Portions of each extract containing approximately 10,000 dpm were spotted on tlc plates (20 × 20 cm glass plates coated with a 0.25 mm layer of silica gel G). The chromatograms were developed twice in the same dimensions in benzene:diethylamine (95:5, v/v) (Golab *et al.*, 1966). Diphenamid and its metabolites were located by fluorescence under short wavelength ultraviolet light and by radioautography. To determine the relative quantities of the parent compound and the metabolites, the radioactive spots were quantitatively scraped into vials of scintillation fluid and radioassayed.

A mass spectrometer was used to confirm identification of diphenamid and its metabolites. To prepare the metabolites for mass spectral identification, the extracts from tomato plants were separated by tlc and the plates were developed as described above. The location of the metabolites was determined by radioautography and by chromatographic comparison with authentic standards. The parent compound and the metabolites (except the one remaining at the origin) were separately removed from the tlc plates, eluted from the silica gel, and introduced into a mass spectrometer *via* a direct probe (Schultz and Tweedy, 1971). An unidentified compound (Compound I in Table II) which had an R_f value between those of MDA and DA was present on all tlc plates and radioautograms. We have not confirmed identity of this compound.

The radioactive material which remained at the origin on thin-layer plates was eluted from the plates with methanol. The methanol eluate then was evaporated under vacuum. The residue was dissolved in 10 ml of 0.1 *N* HCl and extracted three times with 20 ml of diethyl ether. The aqueous fraction was heated in a water bath for 4 hr at 70° C and again extracted with ether. The ether extracts were combined, evaporated to near dryness, and spotted on tlc plates, along with authentic diphenamid, MDA, DA, and DAA. The tlc plates were developed, dried, and exposed to X-ray film. Residual ether was removed from the aqueous fraction under vacuum. The solution was neutralized and portions were enzymatically tested for glucose with a glucose oxidase–peroxidase system (Keston, 1956). These samples were subsequently spotted on tlc plates and chromatographed with sugar standards. The tlc plates were pretreated with sodium bisulfite and activated at 100° C for 15 min. The chromatograms were developed in ethyl acetate:methanol:acetic acid:water (60:15:15:10, v/v), dried, sprayed with aniline hydrogen phthalate, and heated at 85° C until spots appeared.

RESULTS AND DISCUSSION

The amount of ^{14}C-diphenamid removed from the ambient solution by the plants under various light and relative humidity regimes is shown in Table I. Under low light, low humidity, tomato plants removed more of the herbicide from the ambient solution than under the high light, high humidity regime. Radioactivity rapidly increased in the shoots of plants grown under both environmental regimes but was higher in the plants grown under the low light, low humidity regime than in the high light, high humidity regime.

The compound which remained at the origin on thin-layer plates has been tentatively identified as a diphenamid–glucose complex (MDAG) on the basis of the following evidence. A spot was visible on tlc plates of the ether extracts of the MDAG hydrolysate and this spot cochromatographed with MDA in the benzene, diethylamine solvent

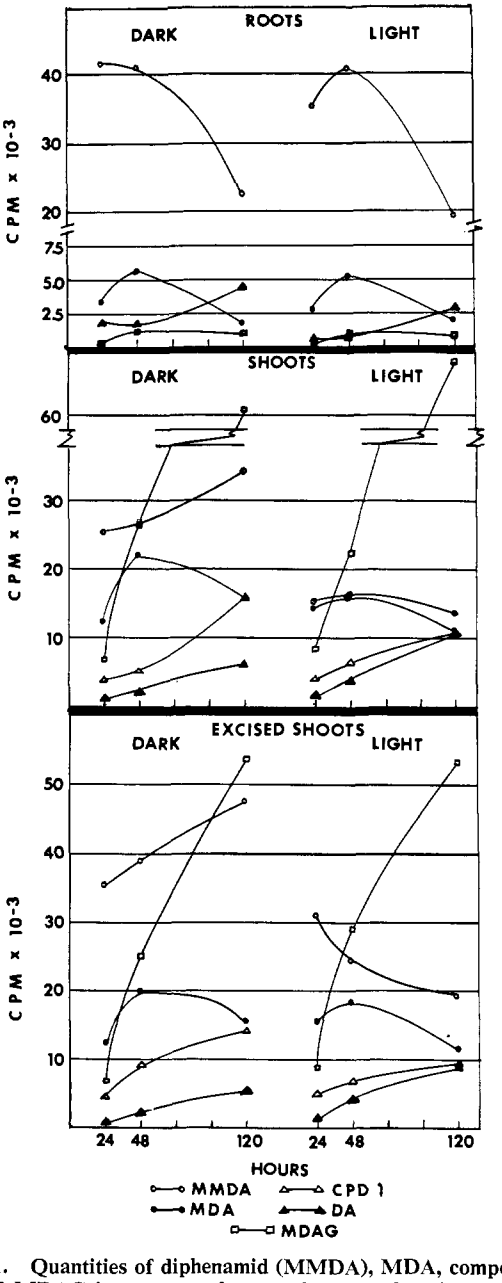

Figure 1. Quantities of diphenamid (MMDA), MDA, compound I, DA, and MDAG in extracts of roots, shoots, and excised shoots of tomato plants grown under low light, low humidity (dark), and high light, high humidity (light) conditions. Values on ordinate have been multiplied by 10^{-3}

Table I. Radioactivity Extracted from Tomato Plants Grown under Different Light and Relative Humidity Regimes

Hours following treatment	dpm/g fresh weight and standard deviation: Low light,[a] low humidity	High light,[b,c] high humidity
	10^3 dpm	10^3 dpm
Roots		
24	49.3 ± 1.91	45.8 ± 8.06
48	57.5 ± 5.00	56.8 ± 4.28
120	32.2 ± 3.02	29.0 ± 1.98
Shoots		
24	58.3 ± 1.27	50.9 ± 2.12
48	97.2 ± 3.96	76.4 ± 3.67
120	155.0 ± 15.48	132.2 ± 22.63
Excised shoots		
24	70.2 ± 21.71	72.8 ± 13.01
48	116.6 ± 23.62	95.6 ± 15.91
120	159.2 ± 5.87	118.0 ± 10.96

[a] Light: 400 ft-candles. Relative humidity: 44 to 50% day, 75 to 85% night. [b] Light: 1500 ft-candles. Relative humidity: 80 to 88% day, 85 to 95% night. [c] All plants were grown under 14-hr day, 10-hr night. Temperature 22 to 24° C day, 13° C night.

system. When the remainder of the hydrolysate was neutralized and portions were tested for glucose, a positive reaction was obtained. A compound which cochromatographed with glucose was also present in the neutralized hydrolysate.

Distribution of Radioactivity in the Extracts. The percentages of diphenamid and metabolites in tomato plant extracts are shown in Table II. The percentage of diphenamid in the roots declined slowly as compared to the metabolites, but in the shoots it declined rapidly from the 24-hr to the 120-hr harvest regardless of the light or humidity conditions. The concentration of diphenamid was always higher in plants grown under low light, low humidity than those grown under high light, high humidity. The percentage of MDA in the roots increased from 24 to 48 hr and then declined. In the shoots, the percentage of MDA was much higher than in roots at the 24- and 48-hr harvests but then declined markedly.

The percentage of Compound I was variable, but in general the percentage increased with time. DA was absent in the roots but was detected in the shoots. The percentage of this metabolite was higher at all harvest periods in the shoots of plants grown under high light, high humidity than in plants grown under low light, low humidity conditions.

The percentages of diphenamid and its metabolites in excised shoots were similar to those found in shoots from intact plants (Table II). At each harvest period plants grown under high light and humidity conditions always contained a lower percentage of diphenamid and a higher percentage of MDAG than those grown under low light and humidity conditions.

Concentration of Metabolites. The concentrations of diphenamid and metabolites extracted from the various tissues are shown in Figure 1. There were no significant differences in the concentration of diphenamid or metabolites in extracts of roots in any of the light and humidity regimes, but there were several differences in the shoot extracts. The concentration of diphenamid increased in the shoots of plants grown under low light, low humidity from the 24- to 120-hr harvest. The DA content of shoots from plants grown under high light, high humidity was nearly twice that found in the shoots of plants grown under the low levels.

The concentration of MDAG extracted at all harvest periods from all plants was nearly the same. However, the quantity from shoots grown under high light, high humidity conditions was 60% of the total quantity extracted but was only 45% of the total quantity extracted from shoots grown under low light, low humidity conditions (Table II).

Since glucose production is dependent upon photosynthesis, the lower rate of glucoside formation in the plants grown under low light, low humidity conditions could result from a lower rate of photosynthesis. A lower rate of photosynthesis may also affect production of the enzyme(s) responsible for demethylation of diphenamid.

The increases and decreases in the concentration of diphenamid and its metabolites in excised shoots at the different harvest periods were similar to those found in the shoots of intact plants although the quantities were different in some instances (Figure 1).

Table II. Quantity of ^{14}C-Diphenamid and Its Metabolites Extracted from Tomato Plants Which Were Grown under Low Light, Low Relative Humidity and High Light, High Relative Humidity[a]

Compound	Time of treatment and light regime: 24 hr, Low light, low humidity	24 hr, High light, high humidity	48 hr, Low light, low humidity	48 hr, High light, high humidity	120 hr, Low light, low humidity	120 hr, High light, high humidity
Roots (intact plants)						
Diphenamid	86%	90%	83%	84%	82%	78%
MDA	8	7	12	11	7	8
Cpd I	5	2	3	3	8	11
DA						
MDAG	1	1	2	2	3	3
Shoots (intact plants)						
Diphenamid	51	35	32	25	26	12
MDA	25	33	26	25	12	9
Cpd I	9	9	7	10	12	10
DA	2	4	3	6	5	9
MDAG	13	19	32	34	45	60
Excised shoots						
Diphenamid	58	48	44	28	35	19
MDA	21	26	18	22	11	11
Cpd I	8	8	11	9	10	9
DA	2	3	2	5	4	9
MDAG	11	15	25	36	40	52

[a] Means of duplicate analyses.

The above data indicate that tomatoes treated with diphenamid and grown under low light, low humidity conditions accumulate higher levels of diphenamid than in plants grown under high light, high humidity conditions. Also, there is less glucoside production in plants grown under the low light, low humidity conditions. Thus, the phytotoxicity of diphenamid to normally resistant tomatoes could be enhanced by low light, low humidity conditions to the point that death of the plants could occur (Lynch and Sweet, 1971).

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