Residues of Maleic Hydrazide in Field-Treated Potatoes

Maleic hydrazide was determined in potato tubers at intervals after field treatment. Levels declined from 8.3 ppm at 3 weeks to 5.3 ppm at 6 weeks and remained constant thereafter. Storage of samples taken 7 weeks posttreatment did not result in changes in concentration for up to 8 weeks. Analysis of fresh or stored samples for the β-D-glucoside and free hydrazine resulted in no detectable residues of either compound.

Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione) is a plant growth regulator used on potatoes and other crops as a sprout inhibitor. The fate of maleic hydrazide has been examined in tobacco where the major soluble metabolite was identified as the β-D-glucoside (Frear and Swanson, 1978). Formation of the β-D-glucoside has also been observed in leaf segments from apple and wheat (Towers et al., 1958). In contrast to these findings, maleic hydrazide was reported as being metabolically stable in corn roots (Noodén, 1970), although the method of extraction may have hydrolyzed any glucoside present.

Hydrazine, a carcinogen in mice and rats (*IARC Monogr. Eval. Carcinog. Risk Chem. Man*, 1974), has been suggested as a plant metabolite of maleic hydrazide (Biswas et al., 1967). While there are no reports in the literature of studies designed to determine its existence in food crops, hydrazine has been found in small amounts in commercial maleic hydrazide (Liu et al., 1974).

The present investigation was conducted to determine by specific chromatographic methods the levels of maleic hydrazide and its β-D-glucoside occurring in potatoes after field treatment. In addition, the possible presence of hydrazine was examined by gas–liquid chromatography.

EXPERIMENTAL SECTION

Crop and Treatment. Potatoes (Katahdin variety) to be sprayed with maleic hydrazide were grown in two parallel 50-foot rows at the Ottawa Research Station, Agriculture Canada, during the summer of 1979. A commercial formulation containing 3.56 lb/gal maleic hydrazide diethanolamine salt was applied at a rate of 6.5 pints/(100 gal acre) 2 weeks after full bloom according to the manufacturers' recommendation.

Three weeks after treatment, the plot was divided into quarters and samples of tubers were removed from each at weekly intervals. The samples were washed to remove adhering soil, homogenized, and frozen pending extraction and analysis for maleic hydrazide, the β-D-glucoside, and hydrazine. Samples of unwashed tubers taken 7 weeks after treatment were placed in paper bags in the laboratory and analyzed at various intervals to study the stability of residues on storage.

Analyses. Maleic hydrazide and its β-D-glucoside were determined by ion-exchange liquid chromatography exactly as described previously (Newsome, 1980).

Hydrazine was determined by a modification of the method of Liu et al. (1974) involving conversion to bis(pentafluorobenzyl)diazene. Potato homogenate (5.0 g) was extracted by mixing with cold (4 °C) 0.01 N HCl for 30 s in a Sorvall Omni-Mixer. Approximately 4 mL of Celite 545 was added and the resulting mixture vacuum filtered on a Buchner funnel containing Whatman No. 1 paper. The filtrate was transferred to a volumetric flask and made to 50 mL with 0.01 N HCl (25 mL). An aliquot (25 mL) was placed in a 50-mL beaker containing 2 M phosphate, pH 5.2 (0.5 mL), and 1% (v/v) pentafluorobenzaldehyde in methanol (2.0 mL) was added. A standard consisting of 50 ng of hydrazine sulfate in 0.01 N HCl (25 mL) was treated similarly and carried through the remainder of the procedure as for the samples. (Caution: because of its carcinogenic nature, hydrazine must be handled by using appropriate precautions). The pH of the standard and sample reaction solutions was adjusted to 3.5. After transfer to a 60-mL separatory funnel and reaction for 30 min, the derivative was extracted by shaking for 1 min with hexane (10.0 mL). As much water as possible was removed, and a few small pieces of dry ice were added to break the emulsion.

A portion (2.0 mL) of clear hexane extract was shaken for 10 min with 10% potassium metabisulfite (8 mL) in a 15-mL centrifuge tube to remove excess reagent. An aliquot (5 μL) of the hexane layer was injected into a gas–liquid chromatograph for quantitation of the bis(pentafluorobenzyl)diazene.

Gas–liquid chromatography was conducted by using a Varian 1400 fitted with a tritium foil electron capture detector and a 6 ft × $^1/_4$ in. glass column packed with 2% butanediol succinate on 80/100 mesh Chromosorb W, HP. Column, injector, and detector temperatures were 180, 190, and 200 °C, respectively. The nitrogen carrier flow rate was 30 mL/min. Under these conditions, the retention time of the derivative was 4 min. With working attenuation (×8), 30 pg of bis(pentafluorobenzyl)diazene resulted in a peak with $^1/_2$ full-scale deflection on a 1-mV recorder. Samples were quantitated by dilution as required and compared with the peak height of that obtained with the 50-ng hydrazine sulfate standard.

Table I. Maleic Hydrazide Residues in Field-Treated Potatoes at Various Intervals after Treatment

time after treatment, weeks	maleic hydrazide found,[a] ppm
3	8.3 ± 1.7
4	8.4 ± 0.7
5	7.5 ± 0.6
6	5.3 ± 0.5
7	4.6 ± 0.5
8	4.1 ± 0.7
9	3.3 ± 0.6
10	4.3 ± 0.5

[a] Values are the means of determinations on four samples ± SE.

RESULTS AND DISCUSSION

The levels of maleic hydrazide found in potato tubers at various intervals after treatment are given in Table I. The initial decline in the level until 6 weeks after treatment may be due to dilution caused by growth of the tubers. Storage for at least 8 weeks does not significantly alter the amount of maleic hydrazide in potatoes harvested 7 weeks after treatment as shown by the data presented in Table II.

The β-D-glucoside of maleic hydrazide was not detected in potatoes sampled from week 3 to week 8 after treatment nor was any found in samples stored for 2- or 3-week intervals. The minimum limit of quantitation was 2 ppm. In tobacco, it has been found that as the level of maleic

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Table II. Effect of Storage Time on Residues of Maleic Hydrazide in Field-Treated Potatoes

duration of storage, weeks	maleic hydrazide found,[a] ppm
2	4.6 ± 0.4
3	3.3 ± 0.7
4	4.7 ± 0.9
5	3.0 ± 0.4
6	4.0 ± 0.7
7	3.9 ± 0.7
8	3.3 ± 0.9

[a] Values are the means ± SE of determinations performed on four samples of potatoes harvested 7 weeks after treatment.

Table III. Recovery of Hydrazine from Potato Fortified with Hydrazine Sulfate

hydrazine added, ppb	hydrazine found,[a] ppb	recovery, %
0	0.2	
5	4.4	88
10	9.0	90
25	24.8	99
100	100	100

[a] Values are uncorrected for the blank.

hydrazide decreased that of the glucoside increased until after 4 weeks both were at similar concentrations (Frear and Swanson, 1978). Thus, if potato metabolized maleic hydrazide in a manner similar to tobacco, measurable residues would be expected to be found.

The recoveries of hydrazine added as hydrazine sulfate to samples of untreated potato are given in Table III. Satisfactory recoveries were obtained from 5 to 100 ppb of added hydrazine. No detectable hydrazine was found in tubers sampled at 3 and 9 weeks after treatment using this method. Similarly, no hydrazine was detectable in potatoes stored for 2- or 6-week intervals.

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A Simple and Rapid Colorimetric Method for Phytate Determination

A rapid colorimetric procedure is described for determination of phytate based on the reaction between ferric ion and sulfosalicylic acid. Determination of the phytate content of a variety of cereals, legumes, and oilseeds demonstrates the simplicity of this method compared to the cumbersome digestion and colorimetric method for measuring liberated phosphorus.

The majority of phosphorus in plant seeds is present as phytate, inositol hexaphosphate (Asada et al., 1969; Erdman, 1979; Oberleas, 1971). The reduced availability of zinc associated with plant protein diets is attributed primarily to binding by phytate (Atwal et al., 1980; O'Dell and Savage, 1960; Shah et al., 1976). Determination of phytate has been based on its precipitation as insoluble ferric phytate in acid solution (Ellis et al., 1977; Heubner and Stadler, 1914; Makower, 1970; Oberleas, 1971; Wheeler and Ferrel, 1971). Harland and Oberleas (1977) eliminated the precipitation step and extracted phytate directly with 1.2% HCl which was then eluted through an ion-exchange resin to separate inorganic phosphorus. The eluted phytate was subsequently digested with concentrated H_2SO_4 and concentrated HNO_3 and the liberated phosphorus measured colorimetrically. The digestion stage required careful monitoring to minimize losses due to incomplete or excessive digestion. This paper reports a simple and more direct procedure for measuring phytate based on the reaction between ferric chloride and sulfosalicylic acid. These reagents were used by Wade and Morgan (1955) to detect phosphate esters separated by paper chromatography.

EXPERIMENTAL SECTION

Materials. Calcium and sodium phytate were purchased from Sigma Chemical Co. (St. Louis, MO). Amberlite IR-120S resin and AG1-X8 resin were obtained from Rohm and Haas (PA) and Bio-Rad Laboratories (Richmond, CA), respectively.

Preparation of Phytate Standard. Calcium phytate was converted to free phytic acid by the method of Angyal and Russel (1969) using Amberlite IR-120S. The free phytic acid was then digested according to the procedure described by Harland and Oberleas (1977) and liberated organic phosphorus measured colorimetrically (Chen et al., 1956). The amount of phytic acid was calculated from the organic phosphorus by assuming that one molecule of phytic acid contained six molecules of phosphorus. Alternately, sodium phytate can be used as a standard since

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