Table I. Gas Chromatographic Analyses of Soy Proteins[a] following Their Reaction with a Dimethyl Sulfoxide Reagent

Sample	Dimethyl sulfide peak height,[b] amp × 10^8
Blank	11.4, 11.1
L-Methionine, 1 μmol	103, 101
Textured soy[c,d,e] 25 mg (contains 1 μmol of methionine)	140, 173
Soy isolate[d,e] 13.1 mg (contains 1 μmol of methionine)	33.4, 32.3

[a] Samples were reacted in 125-mL serum bottles with 1 mL of 6 N HCl and 1 mL of dimethyl sulfoxide, as described in the text. [b] Values are averages of duplicate injections of two separate bottles. [c] The textured soy contained about half starch. In a control assay of 12.5 mg of corn starch alone, the response was no higher than the blank. [d] Analyses of the soy protein samples in 0.5 N HCl instead of 6 N indicated the absence of cysteine (Lipton and Bodwell, 1976a). [e] The PER values were 2.34 for the textured soy and 0.91 for the soy isolate.

chemical determination of nutritionally available methionine, chemically unaltered methionine is assumed to be the only available form so the methods focus on determinations of the methylthio group of the methionine side chain. In the procedure of Pieniazek et al. (1976), the nitroprusside reaction responds only to unaltered methionine side chains and not to sulfoxide, sulfone, or to sulfonium derivatives. The method, however, has the disadvantage, which is common to many other colorimetric procedures, of possible interference from other protein and nonprotein food components. Moreover, the necessity for protein digestion prior to the assay is an added disadvantage.

The procedures of Ellinger and Duncan (1976) and of Finlayson and MacKenzie (1976) are most specific in that reaction occurs only with unaltered methylthio side chain groups and not with methionine sulfoxide, sulfone, or other derivatives with altered sidechains. The cyanogen bromide reagent reacts directly on the intact protein, with no need of any preliminary digestion, and the product of its reaction with the methionyl residues is methyl thiocyanate; the gas chromatographic measurement of methyl thiocyanate is the basis of the assay. Disadvantages of the approach are the toxicity of cyanogen bromide and the overnight reaction time required for release of the methyl thiocyanate. Recently MacKenzie (1977) reported a modified procedure with a reaction time of only 2 h. Use of a sulfur-specific detector for measurement of the methyl thiocyanate might further improve the cyanogen bromide method.

Although the proposed dimethyl sulfoxide reagent for available methionine offers a very simple and rapid chemical determination and does not require prior hydrolysis, further work is necessary to establish the usefulness of this approach. Our earlier study (Lipton and Bodwell, 1976a) indicated that other amino acids did not interfere and that only unaltered methionine reacted under our selected experimental conditions. We pointed out that other reducing substances might interfere in the assay of food samples since the assay measured the dimethyl sulfide reduction product. However, for the assay of samples such as the soy proteins, reducing substances would not interfere. This procedure may be of value for empirical use in assaying protein concentrates for available methionine.

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A Rapid Bioassay for Pesticide Phytotoxicity

A leaf infusion technique which demonstrates low levels of herbicidal activity in chemical compounds was developed. The test is based on determining the differential accumulation of leaf tissue starch as influenced by the phytotoxicity of the applied chemical. The assay is a half-leaf test that can routinely be completed in 6 h using a minimum of equipment.

Early screening of chemical compounds for phytotoxicity is often hindered by the quantities of chemical required and the number of test plants needed. The purpose of this study was to devise a simple, efficient in vivo bioassay for

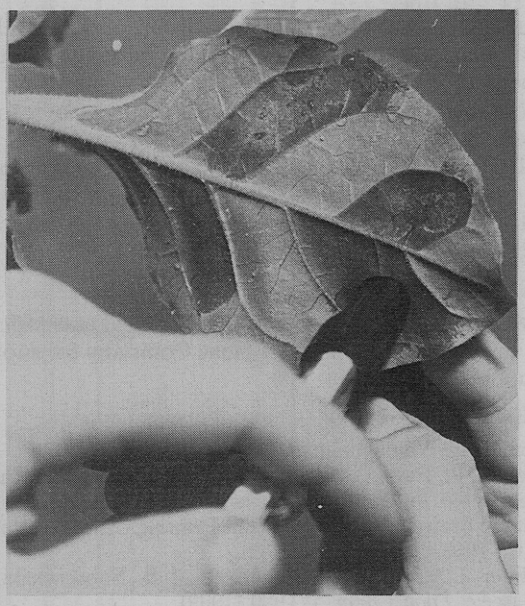

Figure 1. Technique for infusing an intact tobacco leaf with chemical solutions.

small quantities of potentially phytotoxic materials. The bioassay involves hypodermically infusing leaves to irrigate relatively undisturbed leaf mesophyll and parenchyma cells with commercial grades of herbicides, fungicides, and insecticides. After exposure to light, the leaves are tested for inhibition of starch production.

MATERIALS AND METHODS

Six herbicides were selected as representatives of major groups of herbicidal compounds. Four fungicides were chosen as common low phytotoxicity agricultural chemicals, representing three groups of fungicidal compounds. Two insecticides were also tested. The chemicals tested included commercial formulations of 5-bromo-3-*sec*-butyl-6-methyluracil (bromacil 80 W), a substituted uracil; dimethyl tetrachloroterephthalate (dacthal 75 W), a phthalic acid; 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron 80 W), a substituted urea; 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine 80 W), a triazine; isopropylamine salt of *N*-(phosphonomethyl)glycine (glyphosate 41% EC), a substituted glycine; α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine (trifluralin 44.5% EC), a dinitroanaline; manganous ethylenebisdithiocarbamate (maneb 80 W), a carbamate; methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl 50 W), a carbamate; 1-naphthyl methylcarbamate (carbaryl 80 W), a carbamate; 2,4-dinitro-6-(2-octyl) phenyl crotonate (karathane 19.5 W), a dinitro; *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide (captan 50 W), a chlorinated hydrocarbon; and *o,o*-dimethyl phosphorodithioate ester of diethyl mercaptosuccinate (malathion 50 EC), an organic phosphate. Dilutions used were selected to represent rates of minimum phytotoxic reaction and are expressed as ppm of active ingredient (AI).

Young, recently expanded leaves of Turkish tobacco (*Nicotiana tabacum*) at the four- to six-leaf stage were infused with a chemical solution into each of two major vein delimited sections on one side of a leaf. Water was infused into two sections on the opposite side of the leaf. The infusion technique is a modification of the method of Klement et al. (1964) as used for plant pathogenic bacteria. The solution was injected into the back of a leaf with a 2.5 mL syringe fitted with a piece of rubber tubing (Figure 1). Gentle pressure was used to infuse only one vein delimited intercostal section on a leaf, avoiding crossover. The plants subsequently remained in full sun in a greenhouse for 5 h. The leaves were then excised and immediately tested for starch by extracting with boiling acetone and staining with iodine (Kurtz and MacEwan, 1954).

Table I. Inhibition of Starch Production in Tobacco Leaves Infused with Selected Chemicals[a]

Chemical	Rate, ppm of AI				
	0.1	1	10	100	1000
A. Bromacil	F	I	I		
B. Diuron	F	I	I	I	
C. Simazine	N	I	I	I	I
D. Glyphosate		N	N	F	I
E. Trifluralin			N	F	I
F. Dacthal			N	N	N
G. Benomyl			N	N	N
H. Maneb			N	N	N
I. Karathane			F	I	I
J. Captan			N	N	N
K. Malathion			N	N	F
L. Carbaryl				F	I

[a] N, no reaction in test leaves; F, faint reaction, positive but spotty and poor; and I, positive inhibition of starch production.

Rates of 0.1 ppm of AI were used to determine the lower limits of inhibition. Chemical dilutions above 1000 ppm of AI were not used because of the difficulty in infusing concentrated solutions and the resultant mechanical injury.

RESULTS

During the 5-h photosynthetic period, there was no apparent physical injury to the infused leaves at the chemical rates used, except where oil carriers were involved. Absence of starch in an infused area was interpreted as a positive phytotoxic reaction. As seen in Table I, the herbicides (compounds A–F) generally showed a starch inhibition reaction at much lower concentrations than the fungicides (compounds G–J) or insecticides (compounds K–L). With glyphosate, the localized reaction was unsatisfactory; but 5 days after infusion, glyphosate-treated plants showed marked chlorosis and lack of starch in the new leaves. Dacthal did not react to this test.

The fungicides benomyl, captan, and maneb did not exhibit phytotoxicity at 1000 ppm. Karathane showed slight activity at 100 ppm with a strong starch inhibition at 1000 ppm.

Liquid insecticides tested were all very phytotoxic, probably due to the petroleum carriers. The wettable powder carbaryl exhibited slight phytotoxicity at 100 ppm and a stronger reaction at higher rates.

Typical reactions for diuron at 1.0 and 100 ppm are shown in Figure 2. The unstained light-colored area is the site of chemical infusion. The areas of water infusion do not show any interference with starch production. Diuron at 0.1 ppm caused some starch inhibition, generally spotty and localized at the point of infusion, with limited distribution in the remainder of the leaf section. At 1.0 ppm starch production was inhibited throughout the central part of the infused intercostal area. At 100 ppm the diuron which was initially distributed through one intercostal region reacted in adjacent areas along the veinlets (Figure 2b).

DISCUSSION

In trying to differentiate pesticide injury from other causes of crop decline, a leaf infusion bioassay was developed using commercial grades of agricultural chemicals. The technique allows the rapid detection of low level starch inhibiting phytotoxic reactions from chemicals repre-

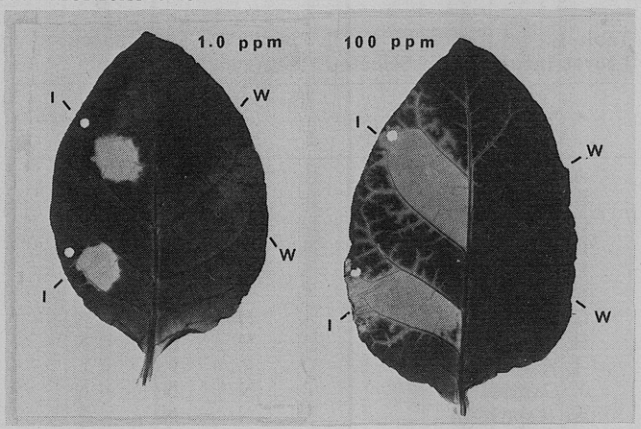

Figure 2. Inhibition of starch formation in tobacco leaves infused with Diuron: I, section infused with chemical; W, section infused with water.

senting several groups of compounds. The 1-day test compares starch production in chemically infused and control portions of a single leaf, using minute quantities of pesticides. The simple technique involves injecting the selected test chemical into a tobacco leaf in the morning before much starch production has commenced and fixing and staining the starch in the afternoon while appreciable leaf storage starch is present. If the injected leaves are left on the plant for several days, the areas exposed to phytotoxic chemicals become chlorotic, precluding the need for starch staining. However, leaving the leaves attached takes longer and is more subject to error in interpretation than the 1-day test.

Those translocatible herbicides with both pre- and postemergence activities (diuron, simazine, and bromacil) (Bayer and Yamaguchi, 1965; Gardiner et al., 1969; Hilton et al., 1974; Hilton et al., 1963; Thomson, 1975) were active in this test at lower concentrations than dacthal and trifluralin which are not readily translocated and do not have significant postemergence activity (Hilton et al., 1974; Probst et al., 1967; Thomson, 1975). The systemic herbicide glyphosate did not exhibit good localized activity but gave a delayed reaction on developing tissue. This is similar to the field reaction observed on several test crops. As expected from their chemical reactions (Lukens, 1971) and the broad variety of plants tolerant to fungicides (Carter et al., 1969), benomyl, captan, and maneb did not generally inhibit starch production in this test. Karathane, a fungicide which also has miticidal properties, did cause tissue collapse and consequent inhibition of starch production at lower concentrations than benomyl, captan, or maneb.

This technique should be useful for the phytotoxicity screening of unknown or experimental chemicals. Only small quantities of materials are required to obtain an in vivo chemical test at rates one might expect to find in the field. If purified chemicals were used, the interfering effects of carriers could be eliminated.

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Comparative Study of the Fluorescent Characteristics of Solutions of Aflatoxins and Palmotoxins in Chloroform

Palmotoxins B_0 and G_0 and aflatoxins B_1, B_2, G_1, G_2, and B_3 were all excited at a wavelength of 365 nm. Their fluorescence emission maxima fell within the wavelength range of 410–430 nm. The fluorescence of aflatoxins and palmotoxins in descending order of intensity is as follows: $G_2 > G_1 > G_0 > B_3 > B_0 > B_2 > B_1$.

There have been reports on the fluorescent characteristics of aflatoxins B_1, B_2, G_1, and G_2 in the solid state (Robertson and Pons, 1968) and in solutions of different organic solvents (Carnaghan, Hartley, and O'Kelley, 1963; Robertson et al., 1965; Bababunmi et al., 1975; Robertson, Pons, and Goldblatt, 1967). On the contrary, there are no reports on the fluorescent properties of palmotoxins B_0 and G_0 which are fluorescent metabolites of *Aspergillus flavus* (U.I. 81) cultured on a medium of palm sap (Bassir and Adekunle, 1968). Also, the fluorescent characteristics of