Analysis for Maleic Hydrazide

PART I. DETECTION AND DETERMINATION IN DRIED GREEN TOBACCO LEAVES AND SUCKERS

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PART II. DETERMINATION AND PERSISTENCE IN SOILS

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A colorimetric method is presented for detecting maleic hydrazide down to 1 p.p.m. in tobacco by analysis of green leaves and secondary growth. An extension of this method for determining maleic hydrazide in soils is also presented. Accumulation of maleic hydrazide is shown to occur in the secondary growth of tobacco. Plant uptake from treated soils is shown to be small. Because of its low persistence in soils, a build-up of maleic hydrazide resulting from plant treatments is highly unlikely.

THE ECONOMICS of tobacco production dictate that sucker or secondary growth must be removed or eliminated from the tobacco plant. Hand suckering, although time consuming and expensive, is the common way in which this is done. The search for a substitute method of suckering led to the use of chemicals, the most successful of which has been maleic hydrazide (MH) (1,2-dihydro - 3,6 - pyridazinedione). Treatment with this chemical not only controlled suckers, but also increased yields, resulting in potentially greater returns per acre. However, differences in the physical and chemical composition of the leaf (*1*, *2*, *11*) resulted in lower acceptability of MH-treated tobacco among manufacturers in general. A scientifically valid and sensitive means of determining MH in tobacco is of prime importance to tobacco growers and processors.

In some instances, this information may be obtained by visual inspection of the growing plant and its deformed sucker growth or by a slight thickening and characteristic color difference in the cured leaf. However, a sensitive chemical method to determine the residue is the only sure way of proving that the chemical has been used. From the practical viewpoint, these data should be available prior to marketing or auctioning of the baled tobacco. To accomplish this, a sensitive and proved chemical method is necessary for the determination of MH in green tobacco leaves and suckers.

It has been reported (*4*) that MH is readily translocated in plants. However, in the case of tobacco, quantitative residue data are lacking for field and greenhouse grown tobacco. On the basis of a report by Smith (*13*) *et al.*, who used C^{14}-tagged MH to indicate the distribution pattern in a tobacco plant, it seemed probable that accumulation would occur in the secondary growth.

A reliable, interference-free, analytical method, valid for the determination of MH in cured tobacco of all types and sensitive to 1 p.p.m., has recently been reported by Hoffman (*9*). The present work was undertaken to find out if this analytical procedure could be extended to green plant material and to obtain reliable residue data on MH in treated tobacco leaves and suckers under both field and greenhouse conditions.

Because of the numerous established uses of MH not only as a growth regulator but also as a herbicide, the possibility exists of a build-up in the soil with subsequent crop contamination and dwarfing of plants. The few studies which have been undertaken along these lines (*6*, *10*, *12*) have been hampered by the lack of a sensitive analytical method for the determination of MH in plants and soils. It has, therefore, been necessary to use biological indicators with abnormally high treatments often over long periods of time. A modified procedure which extends the analytical method (*9*) to the determination of MH in soils is presented, together with a study of MH persistence in various types of soils and of the uptake of MH by tobacco plants from treated soils.

PART I. DETECTION AND DETERMINATION IN DRIED GREEN TOBACCO LEAVES AND SUCKERS

Materials and Methods

For the greenhouse experiments, tobacco seeds, var. Hicks, were germinated in quartz sand, and the seedlings were transplanted to soil in 2-gallon glazed pots. When the flower buds became visible, the plants were topped (four top leaves and flower buds broken off), and then sprayed with aqueous dilutions of the commercial product MH-30. The complete experiment consisted of five treatments with five replicates in each, randomly arranged in a block as follows: untreated check, equivalent of 0.25, 0.75, 1.25, 1.75 pounds active MH per acre.

Since 2.25 pounds of active MH per acre is the dosage usually recommended for effective sucker control on tobacco, small dosages were chosen to allow some sucker growth which could be analyzed and also to ensure that the method of analysis was sensitive enough to detect even low dosage levels. All leaves and suckers were harvested 3 weeks after treatment.

For the field experiments, tobacco, var. Hicks, was grown according to common field practice and, after topping, was sprayed once at the rate of 2.25 pounds active MH per acre. After 3 weeks, representative samples of suckers and top leaves from both treated and untreated plants were removed for analysis.

Leaves and suckers were prepared for analysis by oven-drying at 100° C. and grinding in a Wiley mill. The chemical method used was the same as described for cured tobacco (*9*).

Results and Discussion

No difficulties were encountered in using the analytical method for the analysis of green leaf and suckers. Recoveries of known amounts of MH added to untreated green tobacco showed that the method and standard curve were valid without modification. Results were highly reproducible as shown by values obtained for a treated sample analyzed in quadruplicate: 31.6, 29.0, 30.0, and 29.3 p.p.m.

Table I. Average P.P.M. Maleic Hydrazide in Green Tobacco Grown in Greenhouse (Three Weeks after Treatment)

	Maleic Hydrazide Treatments, (Pounds Active Per Acre)				F	Necessary F	
	0.25	0.75	1.25	1.75		1%[a]	5%[b]
Leaves	4.8	21.4	37.5	48.8	6.69	5.95	3.49
Suckers	30.1	90.6	...	...	...		

[a] Least significant difference 1% 32.8. [b] Least significant difference 5% 24.8.

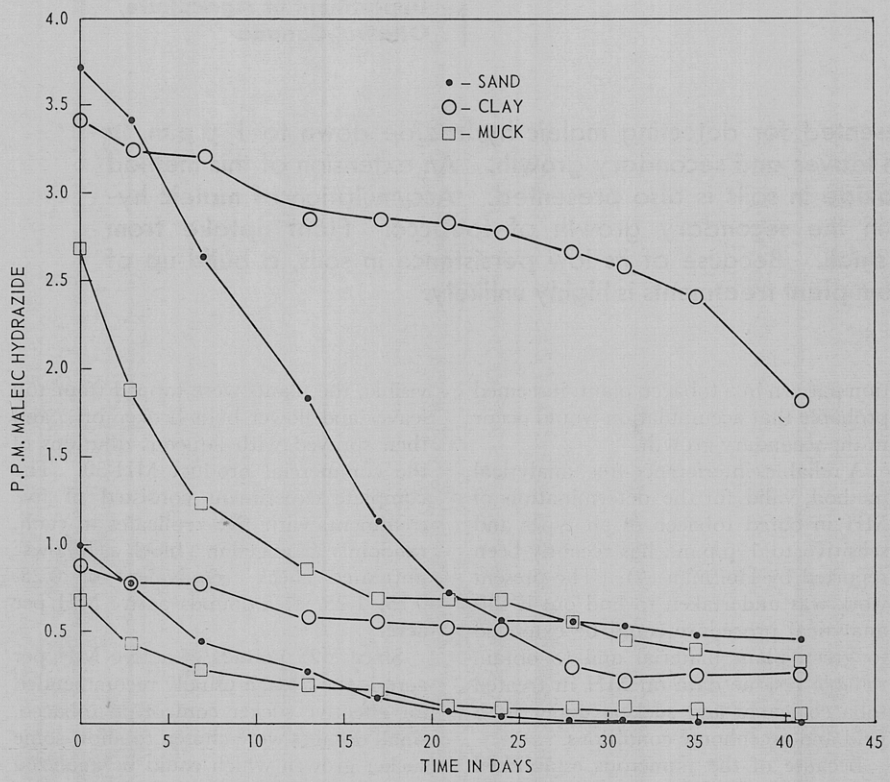

Figure 1. Persistence of maleic hydrazide in soils

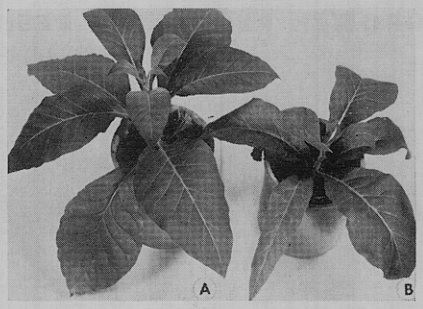

Figure 2. Growth effects from treating soil with maleic hydrazide

The average MH content of green tobacco grown and treated in the greenhouse is shown in Table I. Sucker growth was too small in the case of the two highest treatments to give an adequate sample for analysis. In the case of the 0.75 pound per acre treatment, sufficient sample was obtained only by combining all sucker growth from the five replicates. This exceptional sucker control was probably due to the greenhouse growing conditions which highly favored absorption of MH. This explanation is supported by experiments carried out to determine the rate of absorption of MH by normal plants reported by both Crafts (*3*) and Currier (*5*).

Statistical analysis of the results showed that there were significant differences in the amount of MH found in leaves due to treatment as indicated by the *F* values (*7*). It is evident from the data for suckers that MH tended to accumulate in the secondary growth (suckers), as was expected. Therefore, sucker growth is an excellent diagnostic material for determining the presence of MH.

When tobacco plants were treated in the field at the rate of 2.25 pounds active MH per acre, good, although incomplete, control of suckers resulted. Analysis of a composite sample showed an MH content of 37 p.p.m. in the leaves and 482 p.p.m. in the suckers. Again, there was a large accumulation of MH in the secondary growth.

Chemical testing of green leaves and especially suckers during the growing season evidently is a reliable, sensitive, and practical way to determine before marketing whether tobacco crops have been treated with MH.

PART II. DETERMINATION AND PERSISTENCE IN SOILS

Materials and Methods

Procedure for Determining Maleic Hydrazide in Soils. Place the oven-dried soil (10.00 grams) in a 300-ml., ⅝ round-bottomed flask, add 100 ml. of water, attach a water condenser, and reflux for at least an hour. Centrifuge the boiled sample (a slight cloudiness in extract will not affect procedure) and decant the aqueous portion to a 300-ml. Erlenmeyer flask with spout. Suspend the soil in 50 ml. of hot water with a stirring rod, centrifuge again, and decant into the same Erlenmeyer flask. Add wax and 50 ml. of hydrochloric acid solution and continue with the procedure as described previously (*9*).

To prepare the calibration curve, place 10.00 grams of untreated soil in each of a series of round-bottomed flasks, add known amounts of standard MH solution and proceed as in the analysis of samples. Plot absorbance *vs.* micrograms of maleic hydrazide.

Persistence of Maleic Hydrazide in Soils. Three broadly representative types of soils, previously described (*8*), were chosen for the study—muck, Rubicon sand, and North Gower clay. Each soil was treated to approximate 1 and 4 p.p.m. of MH. The required amount of MH standard solution was mixed with sufficient water to bring the soils up to moisture equivalent, and this solution was uniformly mixed into the soil. The treated soils were then placed in waxed paper cartons and stored at 70° F. and high relative humidity. Cartons were removed periodically over a period of 6 weeks for MH analysis by the method described above.

Uptake of Maleic Hydrazide from Treated Soils. Because of the authors' related work, tobacco was chosen as the test plant. MH standard solution was intimately mixed with a sandy loam soil to give the following concentrations: check, 0.2 p.p.m., 0.5 p.p.m., 1.0 p.p.m., 2.0 p.p.m., and 5.0 p.p.m. On the basis of a 6-inch acre and the admixture of 2.25 pounds of active MH (level commonly applied to tobacco plants for effective sucker growth control), the level in soil would be approximately 1.0 p.p.m. Therefore, the 2.0 and 5.0 p.p.m. treatments represent fairly drastic experimental conditions. Tobacco seedlings, var. Hicks, were transplanted to glazed gallon pots containing treated soil and grown for 2 months. Moisture equivalent was maintained in the soil by periodic additions of water. This technique eliminated

Table II. Average Maleic Hydrazide Content of Treated Sandy Loam Soil after 8 Weeks

Treatment, P.P.M.	Residue, P.P.M.
0.2	0.01
0.5	0.06
1.0	0.11
2.0	0.19
5.0	0.48

run-off and precluded loss of MH by leaching. The harvested leaves were oven-dried, ground in a Wiley mill, and analyzed for MH. Soil from each pot was sampled, oven-dried, and analyzed for MH by the modified method described above.

Results and Discussion

Figure 1 is a graphical representation of the persistence of MH in the three types of soil tested. In all cases, MH content decreased with time. This decrease was very rapid for sand and muck, reaching very low values by the end of 6 weeks. The 1 p.p.m. treatments resulted in negligible residue for sand and muck in this period. Highest residues were present in clay where the decrease was marked but not as rapid as with the other soils.

The MH content of the sandy loam soil on which the tobacco plants were grown in greenhouse is shown in Table II. Approximately 10% of the original amounts added remained in the soil at the end of the 8-week growing period. Again, the decrease in the MH content of the soil was very rapid, although some of this may have been due to uptake by the plants.

The apparent breakdown of MH in the soils tested can be attributed to the action of the various soil microorganisms. The levels of MH used in this experiment were well within the nontoxic range as discussed by Fletcher (*6*). Moreover, Levi and Crafts (*10*) found indications that MH decomposed fairly rapidly in soils under moist, warm conditions. Using much higher levels of MH, these authors also concluded that MH was inactivated slowest in a clay loam. This is similar to the above finding regarding the comparatively slow decrease of MH in clay.

Analysis of the green leaves from the plants grown on treated soils showed the complete absence of any MH residue except in the case of the highest treatment, which was equivalent to at least five times the amount normally recommended for field use. In spite of this high level of treatment, the average residue was only 0.9 p.p.m.

Figure 2 shows a plant from this treatment (*B*) along with an untreated check (*A*). The stunted growth is evident as well as the typical formative effects on the lower leaves. As the plants grew and the MH content in the soil decreased, these effects were overcome and normal growth was resumed.

It can be concluded that there would have to be an enormous amount of MH in the soil before any would be found in the plant. The chemical method for the determination of MH in soils permitted the use of agriculturally effective levels of MH and avoided the danger inherent in biological indicator methods which require extreme experimental conditions.

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HERBICIDE METABOLISM

Formation of a Water-Soluble, 3-Chloroaniline-Containing Substance in Barban-Treated Plants

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BARBAN [4-chloro-2-butynyl *N*-(3-chlorophenyl)carbamate] is the active ingredient of Carbyne (Spencer Chemical Co.), a herbicide used commercially for control of wild oats (*Avena fatua*) in field crops. In the analytical procedure for the determination of barban residues in crop samples, barban is extracted from plant tissues by ethylene dichloride or a similar nonpolar solvent, and hydrolyzed to yield 3-chloroaniline which is then determined colorimetrically (*6*). It was found that ethylene dichloride–extracted plant tissue still contained a substance (or substances) which gave, upon hydrolysis, a positive test for 3-chloroaniline. This substance was not removed by continued extraction of the plant tissue with ethylene dichloride, but could be extracted with water. Apparently some of the barban, which is only 11 p.p.m. soluble in water, had been converted by the plant into a water-soluble, 3-chloroaniline-containing substance. This discovery prompted the assaying of all barban-treated crop samples for the water-soluble, 3-chloroaniline-containing substance as well as for barban, and initiated investigations on its formation and nature. For convenience, this water-soluble, 3-chloroaniline-containing substance which arises in plants treated with barban will be designated as *X*.

Analytical Procedure

The analytical procedure for *X* is based on the method used for the determination of barban (*6*). Barban is extracted from treated plants with ethylene dichloride or a similar nonpolar solvent. *X*, being water-soluble, is not extracted by these solvents and remains in the plant tissue. Essentially the same analytical procedure is used for the determination of barban and for *X*. For the former, the organic-soluble residues are analyzed, while for the latter the extracted tissues are analyzed for their 3-chloroaniline content.

Originally, *X* was determined by re-