on potato chips were carried out with potatoes receiving treatment with a 1% emulsion of CIPC. Table IV shows that measurable amounts of residue were found in these chips. The effect of reducing the CIPC emulsion to 0.5% of active ingredient was then investigated with the results shown in Table II. In this case no measurable residues were found in the potato chips.

In interpreting the residue analyses of the potatoes and potato chips it is recognized that the CIPC may have undergone chemical change or was assimilated and metabolized during storage of the potatoes and their subsequent preparation into chips and therefore may not be detected as such by the analytical method.

Because the ability of CIPC to inhibit the sprouting of potatoes during storage has been effective on an experimental basis, and CIPC residues have always been found with the treated potatoes, the Procurement Section of the United States Army has arranged for chronic toxicity studies on CIPC to be conducted at the University of Virginia. These tests are now in progress.

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Literature Cited

(1) Gard, L. N., Pray, B. O., Rudd, N. G., J. Agr. Food Chem. **2,** 1174–6 (1954).
(2) Gard, L. N., Reynolds, J. L., *Ibid.*, **5,** 39–41 (1957).
(3) Gard, L. N., Rudd, N. G., *Ibid.*, **1,** 630–2 (1953).
(4) Heinze, P. H., Marth, P. C., Craft, C. C., *Am. Potato J.* **32,** 357–61 (1955).
(5) Jones, L. R., Riddick, J. A., *Anal. Chem.* **24,** 569 (1952).
(6) Marth, P. C., Schultz, E. S., *Am. Potato J.* **29,** 268–72 (1952).
(7) Newman, A. S., Downing, C. R., J. Agr. Food Chem. **6,** 352–3 (1958).
(8) Red Dot Foods, Inc., Madison, Wis., private correspondence.

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PLANT GROWTH INHIBITORS

Factors Affecting the Performance of Maleic Hydrazide

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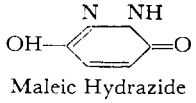
The plant growth inhibitor 6-hydroxy-3(2*H*)-pyridazinone (maleic hydrazide) must be absorbed and translocated to be effective. Studies using tracer, spectrophotometric, and chromatographic techniques showed that maleic hydrazide is stable and nonvolatile, and is efficiently translocated. Absorption is often slow. Experimental techniques made it possible to get quantitative data on variables influencing absorption rate. Light, temperature, and application rate were not critical within the usual range. Plant species and plant condition had significant effects. Relative humidity and formulation were more important. Maleic hydrazide was absorbed poorly from all formulations at low relative humidity. At moderate and high humidities formulation differences were evident. The diethanolamine salt was the most practical of the type absorbed efficiently.

The plant growth inhibitor 6-hydroxy-3(2*H*)-pyridazinone (maleic hydrazide or MH)



Maleic Hydrazide

is applied to plants as a foliage spray under commercial use conditions. To be effective, it must be absorbed and translocated by the plant. Other factors, such as chemical instability or washoff could also affect performance under field conditions.

A program designed to determine the factors which were important to maleic hydrazide performance, and to compare various formulations, was therefore planned. While field results are the final means of determining such effects, the obvious advantages of working under reproducible, controlled conditions indicated the need for laboratory studies.

Experimental

The following techniques were used to study the absorption and translocation of maleic hydrazide.

Absorption Study Technique. Tomato plants (var. Bonnie Best) were grown in individual plastic pots. Plants were selected for uniformity at the time of use, when the plants were about 4 inches tall and had five to six leaves. The watering schedule was such that soil moisture was about halfway between the field capacity (30%) and air-dried levels. The pots were wrapped in polyethylene film to minimize unequal moisture loss from the soil surface. The plants were then sprayed to runoff with a solution of the formulation containing 1500 p.p.m. of maleic hydrazide. This resulted in a deposit of about 1.5 mg. per plant.

For each treatment 19 plants were sprayed. Ten of these were held for 30 minutes to reduce unequal drip off during handling, then cut at the soil surface. The cut plants were washed, two plants per replicate, in 200 ml. of a 100 p.p.m. solution of sodium lauryl sulfate. (The washing technique used had been shown to give complete removal of applied maleic hydrazide in preliminary experiments with several species of plants.) Aliquots of the five wash waters were adjusted to pH 10. This required about one drop of a 40% sodium hydroxide solution. The effect on volume was negligible. The absorbance of the solutions was measured at wave lengths of 303, 328, and 353 mμ using 1-cm. cells with a Beckman DU spectrophotometer (maximum at 328 mμ for salts of maleic hydrazide in water). The readings of 303 and 353 mμ were used to eliminate the interference by using Equation 1.

$$OD_{328} - \left[\frac{OD_{303} + OD_{353}}{2}\right] = N \quad (1)$$

where N is a number proportional to the concentration of maleic hydrazide in the solution. This value is an index of loading rate. If absolute amounts are of interest, construct a standard curve.

The other nine plants were also allowed to stand for 30 minutes, then placed in a controlled temperature-humidity chamber for the absorption period. A 48-hour period was used for much of the work; best accuracy is obtained when the time is such that about half of the maleic hydrazide is absorbed. At the end of the period the plants were washed three at a time in 300 ml. of wash solution, and the solutions were analyzed as above. As at both times the plants are washed in 100 ml. of solution per plant, Equation 2 can be used to calculate the percentage of maleic hydrazide washed off.

$$\frac{N_x}{N} \times 100 = \% \text{ unabsorbed after } x \text{ hours} \quad (2)$$

N = average of the five values for N of Equation 1, from the first set of plants
x = hours allowed for absorption
N_x = average of three values of N of Equation 1, from second set of plants

Data under given conditions were reproducible within 10% in typically replicated tests.

Translocation Studies. For translocation studies, two forms of carbon-14–labeled maleic hydrazide have been used. These were prepared from carbon-14 maleic anhydride, tagged in the 1 or the 2 position. The distinction is not important unless maleic hydrazide is degraded and studies of degradation products are involved. The radioactive material was generally used under conditions such that from 30 to 85% would be absorbed. A radioactivity level of 0.05 μc. per gram of fresh plant weight was satisfactory for radioautographs of most plant parts, using x-ray film and 3 weeks of exposure time.

As maleic hydrazide is not volatile and is readily extracted by water, it was possible to get quantitative data by simply extracting the dried tissues, concentrating the solution, and counting in a windowless flow counter. Frequently, much of the nonvolatile vegetable matter extracted must be separated, if counting is to be done at infinite thinness. Because maleic hydrazide is much more soluble in dilute base than are most of the plant extracts, this separation can often be done by making the extract basic, concentrating and filtering. In some cases a more elaborate cleanup is needed. If the extract from 0.1 gram of tissue can be reduced to infinite thinness on evaporation in a 1-inch counting planchet, a sensitivity of better than 10^{-3} μc. per gram of tissue can be achieved.

For 5-inch tomato plants, an application of 1 mg. of carbon-14 MH at 0.05 mc. per mmole (0.44 μc. per plant) is suitable for both radioautographs and counting work.

Discussion

Factors evaluated for their effect on the performance of maleic hydrazide include possible loss of the chemical from the plant surface before absorption, the absorption rate, and translocation and stability of the plant chemical.

As the biological response to maleic hydrazide is slow and difficult to measure quantitatively, chemical and physical tests were developed to supplement the biological data.

Loss before Absorption. Maleic hydrazide is a high-melting, saltlike solid of extremely low vapor pressure. It is not hydrolyzed in refluxing aqueous sodium hydroxide or in hot sulfuric acid. When a thin film is applied to glass or to the surface of an excised leaf, quantitative recovery can be shown even after 48 hours' exposure to sunlight or to temperatures above those encountered under field use conditions. It seems unlikely that the chemical is lost from foliage by volatility or chemical breakdown. It may be washed off the foliage by rain, but this variable was not a factor under the laboratory test conditions used below.

Absorption. The rate of absorption of maleic hydrazide by plants was studied by applying a known amount of chemical to plants, holding them under controlled environmental conditions during the absorption period, then washing off the remaining chemical by techniques known to give good recovery if no absorption took place (see experimental section). As other likely mechanisms of loss have been eliminated above, the amount not removed by washing should be a useful index of uptake by plants. The term "amount absorbed" is therefore used in this paper to mean the difference between the amount applied and that washed off. In carbon-14 experiments the amount not washed off has been determined directly, and agrees with the data obtained by the indirect method.

Preliminary experiments to establish the relative importance of various factors on the absorption rate of maleic hydrazide were helpful in selecting standard test conditions. While formulations varied in efficiency, the trends discussed below apply to all formulations tested.

Plant Species. Tests on several economically important plants showed that rate of absorption varied moderately with species. For example, tomatoes, nut grass, and quack grass absorbed maleic hydrazide about 70% as fast as tobacco, Johnson grass, and potatoes. For detailed studies the tomato plant was used, as it is convenient to grow and absorbs maleic hydrazide at a suitable rate.

Plant Turgidity. The rate of chemical uptake decreases as plant turgidity decreases even before wilting is apparent. When the plant actually wilts, absorption is severely curtailed. The best correlation with field data is obtained using plants that are not fully turgid, but are far above the wilting point. In the standard test procedure turgidity is controlled by having soil moisture in the pot about halfway between field capacity and the air-dried level. The pots and soil surface are protected from drying by a polyethylene wrapper.

Application Rate. A spray deposit of from 1 to 2 mg. of maleic hydrazide inhibits the growth of 5-inch tomato plants. The absorption rate is approximately independent of the amount applied over the range of 0.5 to 3 mg. per plant, whether plants were treated by over-all sprays or by micropipet applications to one compound leaf. Spray application of about 1.5 mg. per plant was used in most of the work, but on grasses and with carbon-14–tagged material the micropipet technique was used.

Temperature. The temperature at which plants were held during the absorption period had a moderate effect on the absorption of maleic hydrazide. Tomato plants absorbed about 60% as fast at 50° as at 90° F.—75° F. was chosen as standard.

Light. Using the controlled climate facilities, no significant difference in the rate of absorption occurred between plants exposed to an 18-hour photoperiod (1000 foot-candles) and those held for 48 hours in continuous darkness. Standard experiments were run in the dark as there was some tendency for localized differences in temperature to occur, when lights were used.

Humidity. The most important of the environmental effects studied was relative humidity. The absorption rate at 100% relative humidity is two to three times that at 75% and three to five times that at 50% relative humidity for representative formulations.

Formulation. Maleic hydrazide is acidic and only slightly soluble in water. Its salts with volatile amines revert to free maleic hydrazide on the leaf surface. With such formulations absorption was slow under most conditions, possibly because the spray deposit is crystalline and therefore only a small fraction of the chemical is in effective contact with the leaf, and the mobility of the maleic hydrazide anion is low.

The salts of alkali metals and nonvolatile amines were studied in more detail. Table I shows the percentage of maleic hydrazide absorbed from formulations after 48 hours at two humidities (% RH).

All the formulations were absorbed slowly at 75% relative humidity, and differences among formulations were less than the experimental error, except that the potassium salt formulation was less effective than the others.

the potassium (and sodium) salts of maleic hydrazide crystallize on the foliage. The addition of a humectant, such as sorbitol, minimizes crystallization and improves such formulations markedly, as judged by both biological response and absorption data. Similar trends were noted at 100% relative humidity (Table I).

The salts of nonvolatile amines also perform well. The dodecylamine salt, a waxy solid, was absorbed well but the phytotoxicity, cost, and inconvenient solubility characteristics make this formulation less practical. Ethanolamine derivatives give hygroscopic salts which are absorbed well—choline and diethanolamine (DEA) are examples.

Maleic hydrazide formulations based on these salts do not need, and are not significantly improved by, the humectants helpful with the metal salt formulations. Many other additives—e.g., urea, ammonium salts, oils, and stickers such as carboxymethylcellulose—have been evaluated in diethanolamine salt formulations, but none has significantly increased the absorption rate. Enough surfactant to ensure wetting the foliage is helpful, but extra amounts have shown no advantage.

The diethanolamine salt and the potassium salt with sorbitol (K.MH+S) were chosen for detailed study. The plain potassium salt, which had shown poor absorption characteristics in the preliminary work, was included for comparison. All formulations contained a wetting agent and were applied as dilute aqueous sprays. Tests were designed to show the effects of humidity and formulation on absorption rate.

When the data are plotted as per cent absorbed *vs.* time, an exponential curve is obtained. This indicates that absorption is first order—i.e., proportional to the amount remaining on the foliage. This observation has previously been made for several herbicides (*6*). When the data are plotted on inverted semilog paper a straight line is obtained. The slope of the line is the specific absorption rate. If relative humidity is plotted on the third axis of a three-dimensional graph, the response surface for the diethanolamine salt is obtained (bounded by the solid line in Figure 1). The potassium salt–sorbitol formulation shows a similar response surface, only slightly below that of the diethanolamine salt. The simple potassium salt (K.MH) formulation is absorbed less efficiently, as shown by the lower response surface in Figure 1 (broken lines).

A practical measure of formulation efficiency is the time required for a given fraction of the applied maleic hydrazide to be absorbed. Cutting through the three-dimensional plot in a horizontal plane at the 50% absorbed level, the time needed for 50% absorption ($t_{1/2}$) at various humidities can be seen, as in Figure 2. For example, at 75% relative humidity, half of the maleic hydrazide applied as the diethanolamine salt or potassium salt–sorbitol formulations is absorbed in about 3 days. With the simple potassium salt formulation, about 6 days are required. At 100% relative humidity the better formulations have $t_{1/2}$ values of about 1 day, compared with 3 days for the potassium salt formulation.

Table I. Effect of Formulation on Absorption of MH by Plants

(Per cent[a] absorbed in 48 hours)

Formulation	75% RH	100% RH
Potassium salt, K.MH	20	40
Potassium salt + sorbitol, K.MH + S, 1:1	35	65
Dodecylamine salt	40	75
Choline salt	40	80
Diethanolamine salt, DEA.MH	40	80

[a] Average ±10% absolute.

Rapid absorption is very desirable, because these are soluble formulations and are readily washed off by rains. Field results have also indicated that even aside from possible washoff problems, rapid absorption is desirable for best performance.

Fate of MH after Absorption. The rate and pattern of translocation of maleic hydrazide in plants, and its chemical stability within the plant have been investigated.

TRANSLOCATION. Crafts (*3*) has reported that maleic hydrazide moves readily in plants once the active moiety reaches the phloem. In this work the carbon-14–labeled chemical, applied as the diethanolamine salt to one leaf of tomato plants, showed good mobility within the plant. For example, 24 hours after application, about 35% of the absorbed maleic hydrazide had left the treated leaf. Because the chemical was being absorbed gradually, the average time available for translocation from the treated leaf was less than 24 hours. After 4 days, 70% of the absorbed maleic hydrazide had left the treated leaf, 30% being in the young expanding tissues.

Whether carbon-14 maleic hydrazide was applied to the top leaves, bottom leaves, middle leaves, or stem of tobacco plants, a similar distribution pattern resulted. Most of the chemical leaving the treated leaf went to the growing tissues (apical and axillary bud regions) irrespective of the site of application. Two other relative minor effects appeared to be superimposed on the main trend. First, there was a proximity effect, tending to increase the amount of chemical in leaves immediately adjacent to the treated leaf. Second, there was a radial effect tending to increase the amount of maleic hydrazide in the leaves directly above and below the treated leaf. Tobacco leaves spiral around the stem, and the fourth leaf above or below the treated leaf usually was the first one on the same side of the plant. This distribution pattern is consistent with the known vascular pattern of the tobacco plant (*7*).

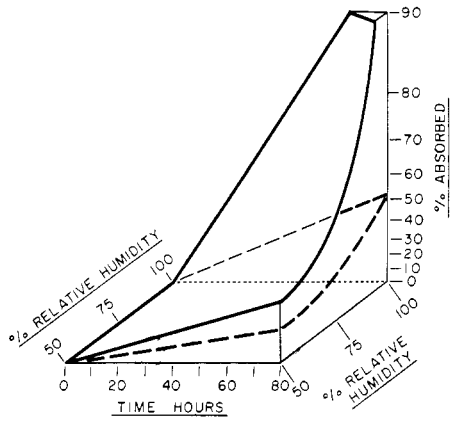


Figure 1. Three-dimensional response surfaces for two maleic hydrazide formulations—% absorbed *vs.* relative humidity and time

——— Response surface of diethanolamine salt of maleic hydrazide, — — — Surface of potassium salt formulation

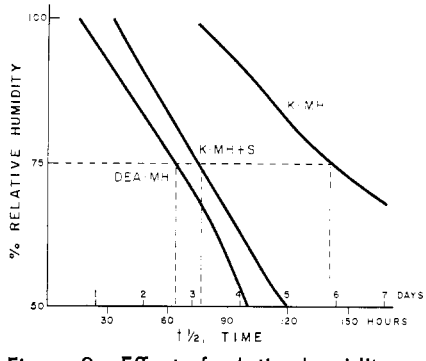


Figure 2. Effect of relative humidity on $t_{1/2}$ (time for 50% absorption) for three formulations of maleic hydrazide

CHEMICAL STABILITY IN PLANTS. The degree of stability of maleic hydrazide inside the plant is not entirely clear. In experiments involving use of carbon-14–labeled maleic hydrazide, most, if not all of the carbon-14 extracted from treated plants has been present as maleic hydrazide. However, not all of the carbon-14 has been accounted for in experiments lasting a week or more.

The carbon-14 not accounted for may be lost as an unextracted complex. Two recent reports show that maleic hydrazide can be bound as a protein complex (*2*) or glycoside (*9*). Alternatively it might degrade to give volatile or nonextractable carbon-14 products. In vitro, maleic hydrazide is much more resistant to hydrolysis than to oxidation or reduction. The possibility of oxidative degradation is supported by Andreae's work (*1*).

The growth inhibiting effect of maleic hydrazide on plants continues for many months after the original chemical application. For example, Crafts (*3*) has reported that Bermuda grass will remain inhibited for over a year. Also, similar results are indicated for other grasses (*4*), potatoes (*8*), and sweet potatoes (*5*). Unfortunately, biological data do not permit differentiation between the actual presence of maleic hydrazide and growth inhibition caused by irreversible changes in the plant, which might persist after the chemical itself has disappeared.

Direct evidence of the persistence of maleic hydrazide in plants can be seen from residue data. By Wood's colorimetric method (*10*), normal residue levels have been found in potatoes and in the roots of turf grasses as long as 8 months after treatment. The residue method is fairly specific for maleic hydrazide, and preliminary isotope dilution and paper chromatography experiments on extracts of plants treated with carbon-14 maleic hydrazide also show the presence of carbon-14 maleic hydrazide in the extracts. Apparently at least part of the chemical in the plant tissues resists breakdown for long periods, and it seems unlikely that instability of maleic hydrazide in the plant is a major factor limiting performance.

Correlation with Field Results

Conclusions based on the laboratory studies were tested under field conditions in two locations. Radishes were sprayed with several formulations at equivalent rates. After harvest the roots were analyzed for maleic hydrazide. Such a procedure measures overall performance, not merely absorption rate. It was felt that if this test ranked the formulations in the same order as the laboratory absorption tests, it would support the idea that absorption was a limiting factor in field performance. As environmental conditions could not be controlled, they were merely recorded. Results are shown in Table II.

Table II. MH Residues

Formulation	Parts per Million: 24 hours, 60 to 100% RH	Parts per Million: 72 hours, 35 to 100% RH
DEA salt	14	21
Choline salt	..	23
Potassium salt + sorbitol	12	16
Potassium salt	10	..
Sodium salt	8	..

These residue data are consistent with the laboratory findings. Further support comes from performance data on potatoes. The diethanolamine formulation (MH-30) performs more consistently than a sodium salt formulation, and sprout inhibition and residue levels correlate very well.

Field results, especially on tobacco, indicate that rapid initial absorption may be desirable. For example, about half of the chemical (applied as the diethanolamine salt) might be expected to be absorbed in 4 days at 50% relative humidity or in 1 day at 100% relative humidity (Figure 2). Even when no rainfall occurs to wash off unabsorbed material, the more rapid absorption appears more efficient. This probably involves many complex variables not considered in the laboratory tests.

Field results on tobacco also support the idea that plants growing in dry soil (presumably under moisture stress) absorb more slowly at a given humidity than plants growing in wet soil under similar conditions.

Thus, while a field testing program is the final means of determining the value of any agricultural chemical or formulation, there are obvious advantages in being able to do the preliminary evaluation under controlled conditions. It is thus possible to examine each factor independently. The general quantitative technique described should be useful for studying factors affecting absorption of other systemic agricultural chemicals.

Literature Cited

(1) Andreae, W. A., *Arch. Biochem. Biophys.* **55,** 584-6 (1955).
(2) Baker, J. E., private communication, 1957.
(3) Crafts, A. S., Currier, H. B., Drever, H. R., *Hilgardia* **27,** 723–57 (1958).
(4) Escutt, J. R., *J. Sports Turf Research Ind.* **8,** 1–5 (1953).
(5) Ezell, B. D., Wilcox, M. S., J. Agr. Food Chem. **2,** 513–15 (1954).
(6) Freed, V. H., Montgomery, J., Research Rept., Western Weed Control Conf. p. 99, March 1957.
(7) Hayward, H. E., "The Structure of Economic Plants," Macmillan, New York, 1938.
(8) Rao, S. N., Wittwer, S. H., *Am. Potato J.* **32,** 51–9 (1955).
(9) Towers, G. H. N., Hutchinson, A., Andreae, W. A., *Nature* **181,** 1535–6 (1958).
(10) Wood, P. R., *Anal. Chem.* **25,** 1779–83 (1953).

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PLANT TISSUE ANALYSIS

Leaf Analysis. Errors Involved in the Preparative Phase

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A critical investigation of the washing, drying, grinding, and storage of citrus and pineapple leaf material, prior to chemical analysis for nutrient elements, is presented. All these preparative steps are subject to relatively large errors. Satisfactory procedures for minimizing the errors are suggested.

After harvesting, plant material is usually subjected to four different preparative steps before the actual chemical analysis is carried out: washing the material to remove surface contamination, drying to stop enzymatic reactions and prepare the material for grinding, mechanical grinding to reduce the material to a state of subdivision suitable for analysis, and final drying to constant weight to obtain a standardized value on which to base the analytical figures. Two other steps are often necessary: storage of the fresh material prior to washing and drying, and storage of the leaf powder prior to analysis.

A careful survey of the literature has revealed a great deal of variation in plant analysis technique, and the newcomer to this field often has great difficulty in selecting efficient methods to suit his purpose. The author, primarily interested in sampling studies for which accurate analytical procedures were essential (*15, 16*), carried out a careful investigation of the complete preparative phase involved in the chemical analysis of citrus and pineapple leaves.

Apparatus

All flame photometric work was carried out on an Eel flame photometer, using "bottle gas" and compressed air.