Maleic Hydrazide

Plant growth regulator

Background Information

In use since the early 1950s, maleic hydrazide (MH) is a first generation synthetic plant growth regulator that is widely used for controlling axillary sucker growth in tobacco. It possesses systemic properties and once applied, typically to the upper half or third of the tobacco plant within 24 hours of topping, penetrates extensively into the tobacco and is actively transported to meristematic tissues where cell division occurs.

Topping and suckering are key practices for increasing yield and producing desired quality in broad leaf tobacco types, such as burley and flue-cured Virginia. Commercial tobacco produces a single stalk terminated by the inflorescence at its apex, which increasingly inhibits growth and physiological activity in the rest of the plant as it develops. Topping, i.e. removal of the inflorescence and some of the apical leaves overcomes this dominating effect and also results in re-direction of assimilate to alternative sinks in the plant. Physiological and growth activity in all relatively young tissues in the remainder of the plant is rejuvenated and ageing is delayed. As a result, leaf weight and size are increased, by as much as 50% and 35% respectively. Root growth is also stimulated. This results in increased production of many of the chemical compounds important to quality. Because topping affects young rather than older tissues, early topping, when the inflorescence is in its early stages of development, has a larger effect than late topping. The primordial cells in leaf axils are also stimulated to grow and form suckers by topping. From an early stage in their growth they progressively exert their dominance and start to inhibit the benefits of topping to the remainder of the plant. To obtain the full benefits of topping it is therefore also necessary to remove suckers as early as possible. This can be done manually, but chemicals, such as maleic hydrazide, provide the most effective and practical way of preventing suckers growing from an early age.

MH inhibits plant cell division, thereby preventing the growth of newly developing suckers, without retarding the elongation of more mature tobacco leaves[1]. MH must be absorbed and translocated to be effective. Other factors, such as chemical instability or wash-off could also affect performance under field conditions[2].

Maleic hydrazide is often used in combination with contacts, C_6-C_{12} fatty alcohols, and/or local systemics such as flumetralin or butralin.

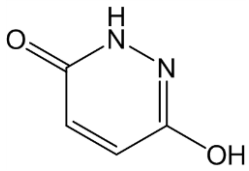
MH, as the free acid, is not used as an agrochemical due to its low water solubility. It is instead formulated as the potassium salt of MH (K-MH; CAS RN [51542-52-0]), which possesses a much higher water solubility (400 versus 4.5 g/L at 25 °C) allowing it to penetrate the tobacco plant more efficiently.

*The original commercial formulation for use on tobacco was **MH-30**, a registered trademark of U.S. Rubber Company (now Chemtura Corporation), which consisted of the diethanolamine salt of maleic hydrazide (DEA-MH). The '30' referred to the formulation containing the equivalent of 30% maleic hydrazide3,4. An unforeseen consequence of using MH-30 was that the non-volatile nitrosamine and suspected carcinogen N-nitrosodiethanolamine was produced during tobacco curing and pyrolysis5. The use of MH-30 was suspended in the early 1980s and N-nitrosodiethanolamine is no longer found in tobacco or cigarette smoke6,7,8.*

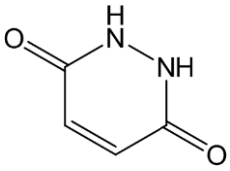
Early investigations into the properties of MH demonstrated that it was a mutagenic agent[9,10,11]. However, further work concluded that the apparent oncogenic activity was due to impurities of hydrazine within MH formulations[12,13]. Current formulations are limited to < 1 ppm hydrazine impurity with no apparent effects (carcinogenic, mutagenic or teratogenic) associated with its use.

The CORESTA Guide No. 1 sets the Guidance Residue Level (GRL) for MH[14].

Properties [15]



Type I



Type II

Figure 1. Maleic hydrazide structure

IUPAC name: 6-hydroxy-2H-pyridazin-3-one (I)
1,2-dihydropyridazine-3,6-dione (II)

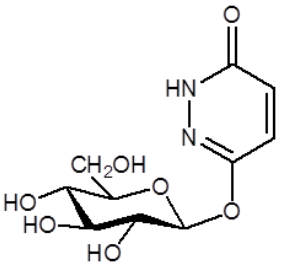
Formula:	$C_4H_4N_2O_2$
Mass:	112.1
CAS RN	I: [10071-13-3] II: [123-33-1]
Form:	White crystalline solid
Solubility:	Water (4.5 g/l at pH 4.3; 25 °C) Methanol (4.1 g/l) Hexane and toluene (<0.001 g/l)
Stability:	Forms salts with alkalis, but is stable in acidic and basic solutions. It is stable to hydrolysis but is decomposed by strong oxidizing agents with release of nitrogen[16,17].
pKa:	5.62
Melting Point:	298 – 300 °C

Maleic hydrazide exhibits keto-enol tautomerization, with proton interchange occurring freely in solution[18].

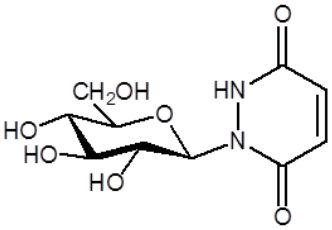
Experimental studies indicate the predominance of the monohydroxy-monoketo tautomer [I] in both the solid state and in solution. The diketo form is also a stable tautomer [II] while the dihydroxy tautomer (not shown) is generally not favoured and of minor importance[19].

Sample Extraction

After application, maleic hydrazide distributes itself throughout the tobacco plant where it can exist as unmodified, or 'free', MH or become bound with cell wall fraction (lignin)[20], or be metabolized with glucose to form glucoside conjugates, *N*-β- or *O*-β-glucoside (Figure 2)[21,22,23].



MH-*O*-β-glucoside



MH-*N*-β-glucoside

Figure 2. Glucosides of MH

Bound and conjugated forms of MH are not thought to participate in physiological responses attributed to MH; therefore such forms may serve as a detoxification mechanism[1].

Levels of 'free' MH decrease after harvesting, curing and storage indicating a gradual conversion to bound and glucoside conjugated forms.

Organic solvents such as methanol have been used to extract maleic hydrazide from plant material. While the glucoside conjugates can be extracted with methanol/water (70:30, v/v)[23], methanol is not able to fully extract bound MH from tobacco.

For the MH glucoside conjugates to be quantitated as MH it is necessary for it to be hydrolyzed with the enzyme ß-glucosidase prior to analysis[24]. However, while ß-glucosidase invertase is capable of hydrolyzing MH-*O*-β-glucoside it has no effect on MH-*N*-β-glucoside[23].

In order to fully extract MH, including that bound to cell walls, aggressive extraction conditions are required. Both caustic and acidic conditions at elevated temperatures have been shown to work well and also have the added benefit of hydrolyzing the glucoside conjugates in the process.

Modes of Analysis

Due to its chemical structure and properties, MH is capable of being analyzed by a range of different analytical techniques.

The earlier procedures involve the hydrolytic reduction of MH to hydrazine by zinc. The hydrazine is isolated by steam distillation and determined colorimetrically as an azine by the addition of *p*-dimethylaminobenzaldehyde. Such colorimetric procedures have been approved by ISO[25] and AOAC[26] as reference and official methods, respectively. The same colorimetric approach was also adopted in the CORESTA recommended method for the determination of MH residues in tobacco[27]. The colorimetric methods, either ISO or AOAC or some variants of them have been widely used in the tobacco industry. However, such procedures have a low sample capacity; they are not specific and susceptible to interferences. Furthermore, the application of these procedures presents a safety issue due to the use of hot alkali[28,29].

Gas and liquid chromatographic procedures have also been widely reported. As MH is generally insoluble in most organic solvents and is nonvolatile it must be derivatized in order to be analyzed by gas chromatography (GC). Numerous MH derivatives have been reported including methyl and Diels-Alder adducts, which allow for a wide range of different detection techniques to be employed such as NPD, FID and ECD[30-36].

The advantage with liquid chromatography (LC) is the ability to analyze maleic hydrazide directly without the prior need for derivatization. In addition, analysis can be performed in either aqueous or organic media eliminating the need for solvent transfer as is common with the majority of GC methods.

Certain methods reported are only able to quantitate free MH[24,32] others can quantitate both free and bound[34,36-38]. However further studies are conducted to confirm this finding.

Analysis *(chromatography and quantitation)*

Examples of the chromatography that can be expected when analyzing MH by GC-FID and HPLC-UV are described in Figures 3 and 4, respectively.

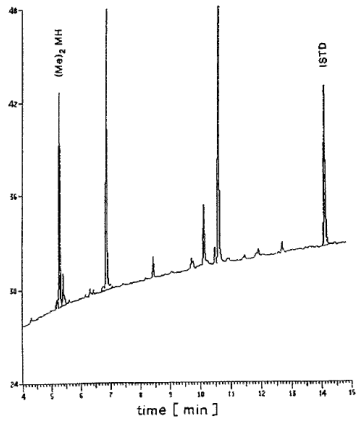


Figure 3. Gas chromatogram of a Virginia tobacco extract containing approximately 200 mg/kg MH[36]

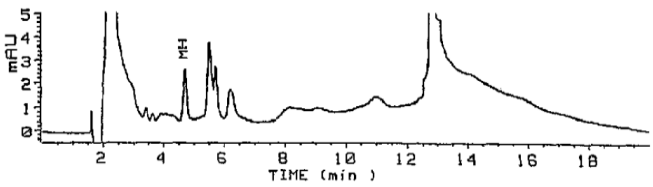


Figure 4. Liquid chromatogram (UV detection) of a Virginia tobacco sample containing approximately 80 mg/kg MH[38]

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