EFFECTS OF APPLICATION METHODS AND RATES OF MALEIC HYDRAZIDE ON THE COMPOSITION OF BURLEY TOBACCO

TOBACCO SCIENCE

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A two-year study was completed to determine the effects of application methods and rates of maleic hydrazide (MH) on the composition of burley tobacco. MH applications were made at 0.5, 1.0, 1.5, 2.0 times the recommended, labeled rate (170mg plant^{-1}) using three application methods (single, split, and reduced-volume application). With an increased rate of MH application, calcium, magnesium, phosphorus, alkaloids, and nitrate levels were decreased, whereas potassium levels were increased. α-4,8,13-duvatriene-1,3-diol increased when MH applications exceeded the recommended rate, whereas β-4,8,13-duvatriene-1,3-diol and solanesol level were not significantly changed. The different application methods affected MH residues, but they had no significant effects on leaf composition. No significant differences were measured in the accumulation of dry matter in leaves from the entire plant.

Additional key words: *Nicotiana tabacum* L., maleic hydrazide, leaf composition, cations, anions, alkaloid, solanesol, diterpenediols.

INTRODUCTION

Topping (decapitation) and sucker control (inhibition or removal of axillary buds) are two key cultural practices for producing quality tobacco (*Nicotiana tabacum* L.). Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione) (MH) is a systemic plant growth regulator that has been the most effective and most extensively used sucker control agent for tobacco in the United States. The influence of MH on the agronomic, physical, and chemical properties of cured tobacco has been investigated extensively (7,10,11,38,44,45,48). In general, MH results in higher yields, equilibrium moisture, sugar and K^{+1}, but decreased nicotine and nitrate when compared to hand-suckered controls. However, studies have not examined changes of leaf components as affected by different application methods and rates of MH. There is an absence of information on the effects of MH application on solanesol and the leaf surface diterpenes, α- and β-4,8,13-duvatriene-1,3-diols (DVT), of burley tobacco. The objective of this study was to determine changes in MH residues and major chemical components in leaves of burley tobacco after different MH application rates and methods were used.

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MATERIALS AND METHODS

Burley tobacco (cv. KY14) was grown using recommended practices at the Kentucky Agricultural Experiment Station Farm (Spindletop) at Lexington, Ky., in 1989 and 1990. The fertilization and production practices for this study have been described (10,11). The following treatments were replicated four times in a randomized complete block design:

A. Control.
1. Hand-suckered control.

B. Single applications of MH with recommended volume of water (392 L ha^{-1} or 20 mL plant^{-1}).
2. 85 mg MH plant^{-1} (0.5x, x = recommended rate of MH).
3. 170 mg MH plant^{-1} (1.0 x).
4. 255 mg MH plant^{-1} (1.5 x).
5. 340 mg MH plant^{-1} (2.0 x).

C. Split-application of MH with recommended volume of water (392 L ha^{-1} or 20 mL plant^{-1}).
6. 85 mg MH plant^{-1} + 85 mg plant^{-1} (0.5 x + 0.5 x).
7. 170 mg MH plant^{-1} + 170 mg plant^{-1} (1.0 x + 1.0 x).
8. 255 mg MH plant^{-1} + 255 mg plant^{-1} (1.5 x + 1.5 x).
9. 340 mg MH plant^{-1} + 340 mg plant^{-1} (2.0 x + 2.0 x).

D. Single application of MH with one-half the recommended volume of water (196 L ha^{-1} or 10 mL plant^{-1}).

10. 170 mg MH plant^{-1} (1.0 x).
11. 340 mg MH plant^{-1} (2.0 x).
12. 170 mg MH plant^{-1} + 170 mg plant^{-1} (1.0 x + 1.0 x).

Green samples from plants that received the recommended rate of MH (treatment 3) were taken at four-day intervals to determine changes in MH residues from time of application to harvest. Each green sample consisted of two leaves from the top stalk position, with one leaf from each of two plants. Midribs of the leaves were removed from the green samples. Lamina tissue was freeze-dried and then ground to pass through a 850-µm screen before residue analysis. Tobacco plants were stalk-cut on the 25th day after topping and air-cured in a conventional barn. Cured leaves were removed from stalks and separated into three leaf positions (top, middle, and bottom) for determination of leaf yields. Ten leaves were randomly pulled from each position for determination of dry matter and chemical analyses. Lamina and midribs were separated, dried at 65°C, weighed, ground to pass through an 850-µm screen, and stored at ambient temperature in the dark before chemical analyses. Stalks were dried in an oven at 65°C for determination of dry matter.

Maleic hydrazide was determined by the AOAC procedure described by Lane (28). Ground tobacco was washed with 9:1 nitric acid:perchloric acid for analyses of potassium, calcium, and magnesium by atomic absorption spectroscopy (24). Soluble phosphate, sulfate, and chloride were determined by ion chromatography with a Dionex model 4000i equipped with AS4A separation column, AG4A guard column and a conductivity detector with background suppression. Total nitrogen was determined, following micro Kjeldahl block digestion, by the phenol-hypochlorite procedure using a Technicon Autoanalyzer (4). Total phosphorus was determined simultaneously on the digested sample using the method of Fiske & Subbarow (18). Nitrate-N was measured with an Automated Technicon Analyzer-System II by Griess reaction after Cd-Cu reduction of NO_3- to NO_2- (46). Individual alkaloids, nicotine, nornicotine, anabasine, anatabine, and myosmine were determined by the method described by Severson et al. (41) and modified by Madsen et al. (31). The values for total alkaloids represented a sum of the individual alkaloids. Solanesol and α- and β-4,8,13-duvatriene-1,3-diols were determined by a GC procedure described by Burton et al. (5) and Severson et al. (40). Nitrite-N was determined by using the Griess procedure reported by Crutchfield & Burton (12).

Data analyses were performed as outlined by the SAS Institute (36). Year and replications were combined as blocks, positions were treated as split-plots and polynomial regression was applied to MH residue *vs* application rates within each application method. Single degree of freedom contrasts were used to test the mean difference of residues from different application methods with same level of active ingredient. LSD was used to compare means.

RESULTS AND DISCUSSION

Agronomic and Physical Characteristics

Across all application rates and methods, MH-treated plants did not have appreciable sucker growth during the first 21 days after topping. A few small suckers developed after 21 days in the treatments with lowest MH rates (85 mg MH plant^{-1}). The values for leaf dry weight and the moisture content tended to be slightly higher and stalk dry weight tended to be lower for MH-treated tobacco compared to hand-suckered tobacco; however, none of these differences was significant (data not shown). Some studies have reported increased yields of MH-treated tobacco over hand-suckered tobacco (38,44,45), but others (1,29,33,35,42) reported that yields either decreased or were not affected.

MH Residues

Following the recommended application rate (170 mg MH plant^{-1}), MH residues of the top leaves decreased with time after application (**Figure 1**), and they best fit the power model (R = 0.99**):

$$MH = 502 \times Day^{-0.84} \qquad (1)$$

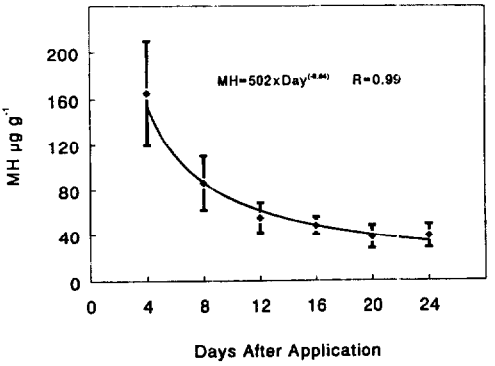
where Day = days after MH application, and MH is in µg g^{-1}.

Residue levels decreased greatly in the first eight days and were in the 30-70 µg g^{-1} range by 12 days after treatment. There was no further significant decrease in MH levels after 12 days. Previous studies showed that lengthening the time between application of MH and harvest usually resulted in lower MH residues (9,14,22,38). Because stalk-cutting of burley tobacco is normally 3-4 weeks after topping, our data indicated that a further delay of harvest to reduce MH residue on burley

tobacco would not be effective when the recommended application rate and method were followed.

MH residues increase with increased amount of application (14,38). Our results confirm these observations, but to compare the different application methods, only treatments of the same application rate are summarized in **Table 1**. Treatments with split-application of MH and with reduced volume of water (high concentration of MH) had higher residue levels than the recommended single application. The effect of split-application has been reported (13) and the high MH residue levels can be explained by the second application being seven days closer to harvest than the single application. Because the potassium salt of MH is very water soluble, it can be assumed that by harvest most of the residue on the leaf surface had been removed by rain and heavy dews. Therefore, the increased MH residue from the single reduced-water application may be a result of more absorption because of its higher concentration in the solution. Furthermore, MH residues in green samples and in cured midribs (data not shown) have the same patterns of residue changes from the different application methods and application rates, which supports the hypothesis of greater MH absorption by the plant from the reduced water-volume application.

Figure 1. Changes in residues of maleic hydrazide (MH) in green leaf samples of the top stalk position of burley tobacco with increased time after application of 170 mg plant^{-1} MH. The analysis was conducted on lamina tissue. Each value is the mean of eight replications from a two-year study and the vertical bars represent the standard errors of the means.



Chemical Composition

Whole-plant averages of selected chemical constituents in cured lamina for all treatments are shown in **Table 2**. The type of MH treatment had a significant effect on Ca^{2+}, Mg^{2+}, K^+, P, NO_3-N, and total alkaloid composition, while no treatment effects were measured for NO_2-N and total N composition. Compared to the hand-suckered control, MH-treated tobacco had increased K^+ (17-34% higher), and decreased Ca^{2+} (9-16% lower), Mg^{2+} (4-16% lower), phosphorus (3-11% lower), total alkaloids (8-26% lower), and NO_3-N (8-28% lower). When the application rate of

Table 1. Maleic hydrazide residues at three stalk positions on air-cured burley tobacco after different application methods at equal application rates.

Treatment			Stalk position		
Method	Water volume	MH rate	Top	Middle	Bottom
	--- L ha^{-1} ---	--- kg ha^{-1} ---	---------- µg g^{-1} ----------		
Single	392	3.36	23.4	24.3	16.9
Single	392	6.72	127.7	58.2	38.4
		Mean	75.6	41.2	27.7
Split	392	1.68+1.68	52.4	34.8	24.7
Split	392	3.76+3.76	185.5	78.7	42.2
		Mean	119.0	56.7	33.5
Single	196	3.36	55.6	27.3	25.5
Single	196	6.72	205.1	104.9	43.1
		Mean	132.4	66.1	34.3
		$LSD_{0.05}$[a]	40.5	ns	ns

[a]LSDs apply to means only.

Table 2. Effects of application methods and rates of maleic hydrazide on selected chemical constituents in air-cured lamina of burley tobacco (whole plant averages).

Treatment			Chemical constituent							
Method	Water volume	MH rate	Ca^{2+}	K^+	Mg^{2+}	P	NO_3-N	Total N	Total alkaloids	NO_2-N
	- L ha^{-1} -	- kg ha^{-1} -	mg g^{-1}							µg g^{-1}
Control	---	---	43.1	24.2	7.6	2.9	5.2	39.2	38.1	2.7
Single	392	1.68	39.3	28.6	7.3	2.8	4.8	40.2	32.5	2.7
Single	392	3.36	38.3	29.7	6.8	2.7	4.5	40.3	35.1	2.4
Single	392	5.04	37.6	29.0	6.7	2.7	4.2	41.4	31.7	2.5
Single	392	6.72	37.2	31.2	6.6	2.7	4.4	41.5	30.0	2.5
Split	392	1.68+1.68	37.0	28.3	6.9	2.7	4.4	40.7	32.3	2.7
Split	392	3.36+3.36	36.4	28.8	6.4	2.7	4.0	40.2	29.0	2.7
Split	392	5.04+5.04	36.5	29.5	6.5	2.7	3.5	41.6	28.8	2.7
Split	392	6.72+6.72	36.1	30.7	6.5	2.6	3.9	41.0	28.5	2.5
Single	196	3.36	38.4	29.7	6.7	2.7	4.0	39.8	33.6	2.6
Single	196	6.72	37.7	32.4	6.6	2.6	3.8	40.2	28.7	2.7
Split	196	3.36+3.36	36.9	30.6	6.6	2.7	3.7	40.1	31.2	2.7
		$LSD_{0.05}$	1.5	2.4	0.3	0.1	0.5	ns	3.1	ns

MH was increased within the same application method, changes of chemical constituents were small or non-significant and inconsistent. Although significant differences for chemical composition were found between the 0.5x rate and higher rates, the largest change was usually between the hand-suckered and 0.5x rate of MH application. F-tests of the single degree freedom contrasts of the changes in chemical composition among different application methods with equivalent rates of the active ingredient were not significant.

Means for each component at each stalk position of MH-treated tobacco and the hand-suckered control are presented in **Table 3**. The changes in composition of lamina from different stalk positions of MH-treated tobacco and the hand-suckered control were very similar. In the cured midrib, each of the constituents was not significantly affected by MH application on a whole plant basis or by leaves within stalk position (data not shown).

The major cations (Ca^{2+}, Mg^{2+}, and K^+) and anions (P and NO_3-N) in leaf lamina from each stalk position are presented in **Table 4**. Like the effect of MH application on MH residue levels, most of the significant differences in ion content were measured from the top stalk

Table 3. Effects of stalk positions on chemical constituents in air-cured lamina of maleic hydrazide-treated and hand-suckered burley tobacco.

Stalk positions	Chemical constituent							
	Ca^{2+}	K^+	Mg^{2+}	P	NO_3-N	Total N	Total alkaloids	NO_2-N
	mg g^{-1}							µg g^{-1}
MH-Treated								
Top	28.6	29.8	5.6	2.6	1.7	44.5	34.9	2.7
Middle	38.0	29.7	6.7	2.8	4.2	42.8	36.1	2.6
Bottom	47.5	28.6	7.9	2.7	6.7	34.3	23.3	2.6
$LSD_{0.05}$	1.0	ns	0.2	0.1	1.1	2.2	2.2	ns
Hand-Suckered								
Top	35.2	23.7	6.9	3.1	2.3	44.2	43.8	2.7
Middle	42.0	25.2	7.3	3.0	5.0	41.1	41.5	2.6
Bottom	49.8	23.8	8.5	2.7	8.3	33.2	28.3	2.6
$LSD_{0.05}$	1.2	ns	0.4	0.2	1.9	2.8	2.5	ns

Table 4. Effects of application methods and rates of maleic hydrazide on major cations and anions in air-cured lamina of burley tobacco.

Treatment			Chemical Constituent														
			Ca^{2+}			Mg^{2+}			K^+			P			NO_3-N		
Method	Water volume	MH rate	Top	Mid.	Bot.	Top	Mid.	Bot.	Top	Mid.	Bot.	Top	Mid.	Bot.	Top	Mid.	Bot.
	L ha^{-1}	kg ha^{-1}	mg g^{-1}														
Control	-----	-----	5.2	42.0	49.2	6.9	7.3	8.5	23.6	25.2	23.8	3.1	3.1	2.7	2.4	5.0	8.1
Single	392	1.68	31.9	39.8	49.2	6.4	7.1	8.3	28.9	27.6	29.1	2.9	3.0	2.5	2.1	4.9	7.9
Single	392	3.36	28.7	38.4	47.9	5.6	7.0	7.9	31.1	29.6	28.1	2.6	2.9	2.6	1.7	4.6	7.0
Single	392	5.04	28.0	37.2	47.6	5.6	6.6	7.9	29.2	28.4	29.4	2.6	2.7	2.8	1.5	4.3	6.8
Single	392	6.72	26.9	37.4	47.2	5.2	6.6	7.9	29.7	30.4	33.5	2.6	2.8	2.7	1.7	4.4	7.2
Split	392	1.68+1.68	28.8	37.7	48.2	5.7	7.0	8.0	29.4	27.5	28.1	2.5	3.0	2.7	1.7	4.3	6.5
Split	392	3.36+3.36	28.5	37.8	44.8	5.3	6.6	7.5	30.6	28.1	27.6	2.6	3.0	2.7	1.3	4.0	6.8
Split	392	5.04+5.04	25.1	36.6	47.0	5.0	6.6	7.8	30.9	28.0	29.7	2.6	2.7	2.7	1.2	3.6	5.5
Split	392	6.72+6.72	26.1	36.7	47.2	5.2	6.4	7.8	30.5	28.6	32.8	2.5	2.6	2.7	1.5	4.0	6.1
Single	196	3.36	28.1	38.7	47.8	5.5	6.7	7.8	29.9	29.4	29.8	2.6	2.8	2.7	1.6	3.9	6.8
Single	196	6.72	27.0	36.5	47.2	5.4	6.5	7.9	32.8	30.9	33.4	2.5	2.7	2.6	2.1	3.9	5.3
Split	196	3.36+3.36	28.6	37.0	47.4	5.6	6.4	7.9	30.9	29.7	31.4	2.5	2.8	2.6	1.4	3.4	6.4
		$LSD_{0.05}$	2.4	1.8	ns	0.4	0.5	ns	4.1	4.1	4.4	0.3	0.2	ns	0.6	0.9	1.3

position. Only the increased K^+ and decreased NO_3-N were significant at the bottom stalk position of the plant. The increased K^+, decreased Ca^{2+}, Mg^{2+}, P, and NO_3-N of MH-treated tobacco confirmed a previous report (48).

The Ca^{2+} content of MH-treated tobacco was 10-28% less at the top stalk position and 5-12% less at the middle stalk position compared to the hand-suckered control, but no significant difference was observed at the bottom stalk position. Lowest Ca^{2+} values were always found in leaves from the higher MH application rates, and there were no significant differences among the different application methods. When lamina and midrib content were combined for all positions, it was estimated that there was an approximate 8-10% decrease of total Ca^{2+} due to MH treatment. Because Ca^{2+} is a relatively large divalent cation with low mobility in the phloem and most of its activity is related to intermolecular linkages in the cell walls and at the plasma membrane, there is a possibility of relatively less Ca^{2+} requirement for structural functions in the leaves at the top of the plant once cell growth is inhibited by MH application. Also, the decreased Ca^{2+} could be a consequence of the antagonistic effect because there was a significant increase of K^+ (2).

The Mg^{2+} content was affected by MH application similarly to Ca^{2+}, with a 7-27% decrease in the top leaves, 3-11% decrease at middle position, and no significant change at bottom of the plant. The estimated total decrease in Mg^{2+} in leaf tissue per plant was 6-15%. Kurvits & Kirkby (27) reported that Mg^{2+} uptake rate was strongly depressed by K^+. Similarly, a K^+ by Mg^{2+} interaction was reported by Hannaway et al. (19). Thus, the decreased Mg^{2+} content may be from increased K^+ and not from a direct effect of MH.

The most abundant cation in tobacco is K^+. It is the most important cation for tobacco burning quality and alkalinity (47), and the one most affected by application of MH. The concentration of K^+ increased 23-39% in leaves from the top position, 9-23% from the middle position, and 16-41% from the bottom position with the addition of MH. The estimated K^+ content for MH-treated tobacco in lamina and midrib of a whole plant was 20-40% higher than that of the hand-suckered tobacco. Similar results were reported by Seltmann & Nichols (38). As a univalent cation and charge carrier of high mobility, K^+ is involved with osmoregulation, long-distance transport, and intracellular pH regulation (43). The role of K^+ in cation-anion balance is also reflected in nitrate metabolism (23). Considering that the cations Ca^{2+} and Mg^{2+} decreased, it is reasonable that there was a considerable increased content of K^+ in MH-treated plants. A portion of the increased K^+ in MH-treated leaves could have come from the K^+ of the applied K-salt of MH. The MH formulation

Table 5. Effects of application methods and rates of maleic hydrazide on total alkaloids at three stalk positions of air-cured burley tobacco.

Treatments			Total alkaloid at different stalk positions		
Method	Water volume	MH rate	Top	Middle	Bottom
	L ha^{-1}	kg ha^{-1}	mg g^{-1}		
Control	----	----	43.8	41.5	28.7
Single	392	1.68	37.9	35.0	24.5
Single	392	3.36	39.1	39.4	26.7
Single	392	5.04	32.5	39.9	22.8
Single	392	6.72	33.4	35.5	21.2
Split	392	1.68+1.68	36.0	38.3	22.6
Split	392	3.36+3.36	33.1	33.0	21.6
Split	392	5.04+5.04	30.3	35.3	20.9
Split	392	6.72+6.72	30.7	34.0	20.7
Single	196	3.36	39.8	37.0	24.0
Single	196	6.72	28.7	34.5	22.8
Split	196	3.36+3.36	33.2	36.9	23.3
		$LSD_{0.05}$	6.3	5.3	4.6

Table 6. Effects of application methods and rates of maleic hydrazide on solanesol and α-&-β-diols in air-cured lamina at the top stalk position of burley tobacco.

Treatment			Chemical constituent		
Method	Water volume	MH rate	Solanesol	α-diol	β-diol
	L ha^{-1}	kg ha^{-1}	mg g^{-1}		
Control	----	----	11.9	0.08	0.04
Single	392	1.68	12.4	0.08	0.04
Single	392	3.36	11.8	0.09	0.06
Single	392	5.04	11.6	0.15	0.06
Single	392	6.72	11.3	0.14	0.05
Split	392	1.68+1.68	11.1	0.09	0.04
Split	392	3.36+3.36	10.6	0.18	0.06
Split	392	5.04+5.04	11.3	0.24	0.07
Single	392	6.72+6.72	9.7	0.18	0.06
Single	196	3.36	12.7	0.14	0.06
Single	196	6.72	10.6	0.18	0.08
Split	196	3.36+3.36	12.0	0.12	0.06
		$LSD_{0.05}$	ns	0.05	ns

contained 5.6% K^+, which means that about 43.9 mg K^+ was applied to one plant at the recommended MH application rate. Even if we assume that all the K^+ from MH application was completely absorbed and 100% remained in the leaf, K^+ from MH application would account for only 5% of the K^+ increase in the leaf. This suggests that increased potassium in MH-treated tobacco was mainly a result of increased K^+ uptake. However, because we did not measure K^+ in the dry roots and because protein-N (calculated as total-N = NO_3-N + alkaloid-N), was increased by MH treatment, another possibility for the increased K^+ concentration was its cycling within the plant for NO_3- transport to the leaves and its subsequent association with protein-N (3).

Phosphorous content decreased significantly in lamina from the top (8-20% decline) and middle (2-13% decline) stalk positions, but no change was measured from the bottom leaves of the plant. Total leaf content of phosphorous (lamina + midrib) from whole plants decreased 3-5%. The function of phosphorous as a constituent of macromolecular structures is most prominent in nucleic acids. If cell growth were inhibited by MH, less phosphorous would be required in the younger leaves at the top of the plant and consequently less would accumulate (21).

Nitrate-N content decreased 5-48%, 5-35%, 3-28% at the top, middle, and bottom positions of the plant, respectively. Because total-N was not affected by MH treatment and total alkaloid decreased, these data suggest that other reduced forms of N compounds (protein) must have increased. Douglass et al. (16) reported that *in vitro*, nitrate reductase activity was higher in roots and lower in leaves of MH-treated seedlings compared to control seedlings. If the same were true *in vivo*, there would be less nitrate in the leaves, as observed in this experiment. Also, Douglass et al. (15) reported that nitrate uptake rate expressed as Nitrate-N per gram dry weight of roots was equivalent for MH-treated and untreated plants. Consequently, similar total-N values were expected and changes in reduced-N forms were affected. Concentration of other anions, SO_4^{2-}, Cl^-, and NO_2^- in the lamina were not affected by MH treatment (data not shown).

In this study, total alkaloids of MH-treated tobacco decreased at all positions compared to the hand-suckered control (**Table 5**). The decreased alkaloid contents ranged 9-34%, 4-20%, and 5-29% for the top, middle, and bottom leaf positions, respectively. However, no significant effects were measured for application method at equal application rates. These results are in agreement with published studies (38).

The solanesol content (**Table 6**) in top leaves of MH-treated tobacco ranged 9.0-12.7 mg g^{-1}, which was not significantly different from the

hand-suckered control (11.9 mg g^{-1}). As the major terpenoid in tobacco, solanesol concentration is influenced by genotype, plant population, soil moisture, and topping (5,37). These data indicate that MH application had no appreciable influence on solanesol accumulation.

The diterpenes, α- and β-4,8,13-duvatriene-1,3-diols (DVT), are present in the cuticular wax of tobacco leaves, and their degradation or pyrolytic products have distinctive flavor and aroma properties that affect tobacco quality (17,39). α-4,8,13-duvatriene-1,3-diol levels increased significantly on the top leaves of MH-treated tobacco when MH application rates exceeded the recommended level **(Table 6)**. However, β-4,8,13-duvatriene-1,3-diol content on the leaves from the top stalk position ranged from 0.04-0.08 mg g^{-1} and was not significantly affected by MH application. These observations are in contrast to those reported by Goins et al. (20) for green flue-cured tobacco. Their results were reported on mg cm^{-2} basis, whereas our values are based on mg g^{-1} basis in cured tissue. Because DVT are the most prominent terpene diols in benzene surface washes of tobacco leaves (8,34), they could be influenced by genetic factors, cultural practices, and environmental factors during the growing season (30,32). Keene & Wagner (26), Kandra & Wagner (25), and Wagner (49) suggested that CO_2 and monosaccharides produced in trichome heads or supplied by other epidermal cells were the major precursors for DVT biosynthesis, which primarily occurs in the glandular trichome heads. The inhibition of the translocation of ^{14}C from a single leaf exposed to $^{14}CO_2$ by maleic hydrazide and the increased sugar content by MH application have been reported (6). Together these data imply that the increased DVT by high MH application rate was caused by increased biosynthesis, because direct injury or change of leaf surface property by MH were not observed (13).

In conclusion, comparison of data from all 12 treatments across two years indicates that MH application did not significantly alter dry matter accumulation and equilibrium moisture of air-cured burley tobacco. Split application and reduced water-volume application of MH significantly increased MH residues with the same level of sucker control and there was no significant change in chemical composition compared to the recommended rate of MH application. MH application decreased calcium, magnesium, phosphorus, alkaloids, nitrate levels, whereas potassium levels were increased in cured burley tobacco. α-4 8,13-duvatriene-1,3-diol content in the leaves from top stalk position of MH-treated tobacco increased significantly when MH application rates exceeded the recommended level, while β-4,8,13-duvatriene-1,3-diol content and solanesol levels were not significantly changed.

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