

CARRYOVER OF SOIL-APPLIED HERBICIDES ON FLUE-CURED TOBACCO

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Paper Accepted on 18 January 2010

Abstract

An outbreak of a tobacco leaf disorder characterized by interveinal chlorosis of lowermost leaves was recorded for the first time in fields on the Beau Champ Sugar Estate in October 2003. Given that tobacco is grown on sugar cane rotational lands, herbicide simulation trials were conducted from 2005 to 2007 at Réduit, Richelieu and Beau Champ to determine whether the residual effects of one or more of the pre-emergence and post-emergence herbicides commonly used in sugar cane might be responsible for the characteristic chlorotic symptoms. On all three sites, symptoms of interveinal and marginal chlorosis and necrosis of leaves were reproduced following tobacco transplantation in plots previously sprayed with either Karmex Flo, Karmex Flo + Velpar, Atrazine + Velpar + Terbo, Tebusan, Garlon, DCMU or Tebusan + DCMU. Such symptoms could thus be attributed to carryover of herbicides of the phenylurea (Tebusan, DCMU and Karmex Flo), pyridine (Garlon) and triazinone (Velpar) families. Since the carryover effects of these herbicides reduced tobacco yield and quality, growers are henceforth recommended to avoid fields sprayed with these herbicides during the last or two-last sugar cane cropping cycles.

Keywords: Flue-cured tobacco, herbicides, interveinal yellowing, leaf disorder.

**For correspondences and reprints*

1. INTRODUCTION

Tobacco, a valuable cash crop in Mauritius is an important source of earning to some 320 flue- cured and air- cured growers (Tobacco Board, 2006). It is usually cultivated on sugar cane rotational lands available from sugar estates. An outbreak of a tobacco leaf disorder was recorded for the first time on 15th October 2003 in fields of Beau Champ Sugar Estate (BCSE) on 2 commercially exploited flue-cured tobacco varieties RG 13 and Speight G28. Characteristic symptoms of the leaf disorder were interveinal yellowing, marginal chlorosis resulting in interveinal necrosis of leaves. The disorder was apparent on lower leaves and progressed on upper leaves until the whole plant was completely affected. Such disorder was again observed in successive tobacco cropping years 2004, 2005 and 2006 in the same factory area with level of incidence between 10 to 70% over 60 hectares (ha) planted each year. The disorder was however not encountered in other growing regions on the same varieties. In 2006, similar leaf symptoms were observed on food crops (creepers, tomato and beans) in the same factory area over an area of 40 ha. The level of incidence ranged between 30 to 100% (AREU, 2006).

Investigations carried out from October 2003 to December 2004 on the possible causes (biological, edaphic, agronomic, nutritional and physiological) did not reveal any association of insects, viruses, bacteria, fungi, quality of irrigation water or soil nutrient imbalances with the disorder. Pot trials indicated that the characteristic symptoms did not progress further in affected seedlings when grown in soils of Réduit contrarily to those grown in soils of Beau Champ. At that point in time, a soil toxicity hazard specific to the Deep River Beau Champ locality was found to be the most plausible explanation to the occurrence of the disorder (AREU, 2005). Given that tobacco is normally planted on sugar cane rotational lands, it was hypothesized that prior to release, these lands might have been sprayed with herbicides to ensure good weed control.

In this context, a study which included herbicide simulation trials was undertaken to determine whether the leaf disorder on tobacco was due to carryover of one or more of the pre-emergence and post-emergence herbicides commonly used to control weeds in sugarcane, to determine which herbicide(s) or family of herbicides was/were kept responsible, and to assess the carryover effects on tobacco yield and quality.

The work reported in this paper is a pioneer in the local context and presents the findings and recommendations of the leaf disorder problem on flue-cured tobacco.

2. MATERIAL AND METHODS

2.1 Herbicide simulation trials

A two-year study was undertaken at Réduit Crop Research Station (CRS), Richelieu CRS and Beau Champ from January 2005 to January 2007. At both Réduit and Richelieu CRS, fields that have not been sprayed with herbicides commonly used in sugar cane fields for the last two consecutive years were selected and single plot experiments were used, each plot consisting of 3 rows of 6 m length. At Beau Champ, the experimental design was a randomized complete block with three replicates, and each plot was made up of 6 rows of 9 m length. The recommended rates of herbicides in sugar cane and their respective rates applied during the simulation trials from 2005 – 2007 are furnished (Table 1). Details of each trial are described:

Table 1: Recommended rates of herbicides in sugar cane and their respective rates applied during the simulation trials from 2005 – 2007.

Herbicide treatment	Recommended rate for sugar cane (kg. a.i. ha ⁻¹)	Dosage rate (% of recommended) (kg. a.i. ha ⁻¹)		
		½	¼	⅛
Atrazine (Gesaprim)	4.8	2.4	1.2	
Goal (Oxyfluorfen)	1.6			
Terbo (Terbuthylazine)	1.8			
Velpar (Hexazinone)	2.4			
Karmex Flo (Diuron)	3.0	1.5	0.75	
Garlon (Trichlopyr)	1.2	0.6	0.3	0.15
Tebusan (Tebuthiuron)	2.6	1.3	0.65	0.325
DCMU(Diuron)	2.4	1.2	0.6	0.3
DMA6 (2,4 D amine salt)	2.6			
Atrazine + Goal		2.4 + 0.8	1.2 + 0.4	
Atrazine + Goal + Terbo		2.4 + 0.8 + 0.9	1.2 + 0.4 + 0.45	
Atrazine + Velpar + Terbo		2.4 + 1.2 + 0.9	1.2 + 0.6 + 0.45	
Karmex Flo + Velpar		1.5 + 1.2	0.75 + 0.6	
Tebusan + DCMU	2.6 + 2.4			

Source: (Rocheouste, 1967)

2.1.1 Trial 1 (Réduit CRS)

A trial was conducted at Réduit CRS from March to July 2005 over an area of 144 m². Four pre-emergence herbicides: Atrazine (Gesaprim), Goal (Oxyfluorfen), Karmex Flo (Diuron) and Velpar (Hexazinone) and one post-emergence herbicide Terbo (Terbuthylazine) were used either alone or in combination. They were specifically chosen because sugar estates, in particular Beau Champ, carry out a second application of these herbicides followed by a third one, if required, 5 - 7 months before sugar cane harvest. They were applied in tobacco furrows on 14th May at two rates: ½ and ¼ recommended rates (Table 1). It was assumed that at these two rates, the herbicides in the soil would be at a level sufficient to trigger the symptoms of interveinal yellowing as noticed in planters' fields.

Six week-old tobacco seedlings of the variety RG 13 were then transplanted at 3 time intervals: 0, 24 and 48 days after herbicide application (DAHA). The delayed transplantings were meant to allow enough time for the herbicides to percolate to the root zone and thereby cause the desired symptoms.

2.1.2 Trial 2 (Richelieu CRS)

A second trial was laid down at Richelieu CRS from October 2005 to March 2006 over an area of 600 m² using herbicides currently used by BCSE, namely Tebusan (Tebuthiuron) Karmex Flo (Diuron) and Garlon (Trichlopyr). This trial was meant to confirm the results from Réduit, by keeping the same methodology, but under a silty clay - clay soil type as opposed to a silty clay soil at Réduit (Parish and Feillafé, 1965). The three herbicides were applied in tobacco furrows on 11th October 2005 at ½, ¼ and 1/8 recommended rates (Table 1). Tobacco seedlings of the varieties K326, RG 13 and Speight G28 were transplanted at two time intervals: one week and one month after herbicide application (AHA). The inclusion of the three varieties was meant to assess their reaction to the herbicides.

2.1.3 Trial 3 (Beau Champ)

Concurrently to the trial at Richelieu CRS, a third experiment was set up at Beau Champ to simulate conditions as per recommended practice in sugar cane fields; that is, to apply herbicides at recommended rates during the last sugar cane ratoon crop eight months before harvest in July. Following harvest, the land is disc-ploughed to allow tobacco cropping in August. Hence, it takes approximately nine months from time of herbicide application to start of tobacco transplantation. Following cane uprooting at the last ratoon, a plot of land of 1000 m² was selected. In October 2005, the

herbicides Atrazine, DCMU, Tebusan, DMA6 (2, 4 D amine salt) and (Tebusan + DCMU) were applied in tobacco furrows as per the recommended rate in sugar cane (Table 1). The tobacco plot was then left fallow until seedlings of the variety RG13 were transplanted nine months AHA. In all three experiments, a no-herbicide plot was used as the control. Standard or recommended tobacco cultivation, fertilization, insect control and harvest practices were followed (ANON, 1990). The crop was maintained weed-free by hand-hoeing.

Percentage of seedling mortality and symptoms of interveinal yellowing, marginal chlorosis and interveinal necrosis were assessed visually. Plants were considered chlorotic if there was any visible yellowing of interveinal leaf tissue or leaf margins and necrotic if there was any visible necrosis. Overall injury which included chlorosis, necrosis and plant vigour was estimated visually on a scale of 0 = no injury to 100 = complete death (Skroch and Sheets, 1977). Observations of the number of chlorotic or necrotic plants were converted to a percentage of the total plants for presentation.

At Beau Champ, data was also recorded on growth parameters. These were compared to a nearby healthy crop from a field at Ernest Florent with no previous history of sugar cane cultivation thus implying that pre-emergence and post-emergence herbicides commonly used in sugar cane fields were not applied. Observations on green and cured-leaf yields were collected from three fields each at Beau Champ and Ernest Florent to assess the difference in yield and quality between affected and healthy tobacco crops.

Data on incidence of foliar chlorosis, growth and yield of tobacco were subjected to analysis of variance and treatment means were compared with either Least Significance Difference (LSD) or Duncan Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

2.1.4 SOIL AND LEAF ANALYSIS

In 2006, based on the area planted, a given number of fields of the Beau Champ region showing the leaf disorder symptoms were selected. In each of the selected fields, 5 random soil samples each of depths 0-10 cm, 10-20 cm and 20-30 cm were taken with a round auger of 10 cm diameter. For each soil horizon, the samples were thoroughly mixed to produce one single composite sample. Entire leaf samples exhibiting the characteristic interveinal yellowing were also collected at different plant positions and made up to 1 kg of fresh weight. Both soil and leaf samples contained in liquid CO₂ under refrigerated conditions at -18 °C were sent to the Groupement Interregional de Recherches sur les Produits Agropharmaceutiques (GIRPA) laboratory, France for multi-residue

herbicide analysis, in particular Diuron, Tebuthiuron and Trichlopyr. This was meant to support the findings of trials at Réduit, Richelieu CRS and Beau Champ.

3. RESULTS AND DISCUSSION

3.1 Herbicide simulation trials

3.1.1 Trial 1 (Réduit CRS)

At the first two planting dates (0 and 24 DAH application), 10- 80% of seedlings died within 13 days of planting, while 100% mortality was observed after 20 days, with treatments Karmex Flo, (Karmex Flo + Velpar) and (Atrazine + Velpar + Terbo) at both ½ and ¼ recommended rates. The incidence of mortality was significantly higher when Karmex Flo and Velpar were applied in combination. Tobacco seedlings were, however, healthy in the no-herbicide control and the remaining herbicide treatments (Table 2).

Table 2: Percentage mortality of seedlings after 13 and 20 days at the first two planting dates (0 and 24 DAHA) with different herbicide treatments at the Réduit CRS in 2005.

Herbicide treatments	Mortality of seedlings (%)							
	Planting Date (days after herbicide application)							
	0				24			
	Observation Date (days after planting)							
	13		20		13		20	
	Rate of application (% of recommended)							
	¼	½	¼	½	¼	½	¼	½
Atrazine	0	0	0	0	0	0	0	0
Atrazine + Goal	0	0	0	0	0	0	0	0
Atrazine + Goal + Terbo	0	0	0	0	0	0	0	0
Atrazine + Velpar + Terbo	30	30	100	100	50	20	100	100
Karmex Flo	80	80	100	100	30	10	100	100
Karmex Flo + Velpar	100	100	100	100	70	70	100	100
No herbicide	0	0	0	0	0	0	0	0
LSD 0.05	10.9	10.9			18.4	6.1		

At the third planting interval (48 DAHA), interveinal yellowing of leaves

became apparent shortly after transplanting with treatments Karmex Flo, (Karmex Flo + Velpar), and (Atrazine + Velpar + Terbo) (Table 3). The incidence of foliar chlorosis was significantly higher with Karmex Flo at the $\frac{1}{4}$ recommended rate while at the $\frac{1}{2}$ recommended rate, the incidence was significantly higher when Karmex Flo and Velpar were applied in combination. Again, as observed in fields on the Beau Champ Sugar Estate, the symptoms progressed from the lower to the upper leaves and within one week the leaves turned necrotic. These symptoms were however absent in Atrazine, (Atrazine +Goal), (Atrazine +Goal+Terbo) and the no- herbicide treatments (Table 3).

Table 3: Percentage of plants exhibiting interveinal foliar chlorosis 48 days after herbicide application of different herbicides at the Réduit CRS in 2005.

Herbicide treatment	Incidence (%) of foliar chlorosis 48 days after herbicide application	
	$\frac{1}{4}$ recommended rate	$\frac{1}{2}$ recommended rate
Atrazine	0	0
Atrazine + Goal	0	0
Atrazine + Goal + Terbo	0	0
Atrazine + Velpar + Terbo	20	20
Karmex Flo	50	40
Karmex Flo + Velpar	40	60
No herbicide	0	0
LSD 0.05	9.8	10.7

3.1.2 Trial 2 (Richelieu CRS)

3.1.2.1 1st planting interval

For the 1st planting interval (1 week AHA), complete death of seedlings was observed 3-5 days after transplantation with Garlon only at $\frac{1}{2}$ and $\frac{1}{4}$ recommended rates. Since interveinal leaf yellowing followed by necrosis was so rapid and were not visible, the incidence was thus rated as 100 percent.

Interveinal yellowing was apparent on the leaves 42- 45 days AHA with Tebusan and DCMU at all 3 rates and with Garlon at $\frac{1}{8}$ recommended rate. At the latter rate, the incidence of interveinal foliar chlorosis was significantly higher with DCMU irrespective of varieties (Table 4). The higher incidence at the $\frac{1}{8}$ recommended rate compared to the $\frac{1}{4}$ rate with DCMU could be ascribed to the relatively faster uptake of the herbicides by capillarity due to accumulation of water around the root system caused by defective dripper lines.

Table 4: Percentage of plants with foliar chlorosis at the 1st planting interval (1 week AHA) with different herbicide treatments at $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ recommended rates at the Richelieu CRS in 2005.

Herbicide treatment	% incidence of interveinal foliar chlorosis								
	RG 13			K326			SPG 28		
	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$
Tebusan	11.0	18.0	27.0	15.0	20.0	26.0	14.0	16.0	30.0
Garlon	100	100	42.0	100	100	45.0	100	100	40.0
DCMU	72.0	39.0	80.0	65.0	41.0	82.0	68.0	36.0	82.0
No herbicide	0	0	0	0	0	0	0	0	0
LSD 0.05	7.2	2.7	7.5	4.1	4.6	8.4	6.3	0.99	7.6

3.1.2.2 2nd planting interval

Similarly, at the 2nd planting interval (1 month AHA), the highest 2 rates of Garlon ($\frac{1}{2}$ and $\frac{1}{4}$) were lethal to the tobacco plants at 3- 5 days after transplantation. The characteristic interveinal yellowing was observed on lower-most leaves 67- 70 days AHA (that is 37- 40 days after transplanting) with Tebusan and DCMU at all 3 rates and with Garlon at the $\frac{1}{8}$ recommended rate. The incidence of interveinal foliar chlorosis was again significantly higher with DCMU at the $\frac{1}{8}$ recommended rate (Table 5).

At both planting intervals, interveinal necrosis is followed 5 to7 days after any visible yellowing of the leaf tissue or leaf margins. All 3 varieties were susceptible to the herbicides. The crop stand in the no-herbicide treatment was healthy.

Table 5: Percentage of plants at the 2nd planting interval (1 month

AHA) showing interveinal foliar chlorosis after different herbicide treatments at 1/2, 1/4 and 1/8 recommended rates at the Richelieu CRS in 2005.

Herbicide treatment	% incidence of symptomatic plants								
	RG 13			K326			SPG 28		
	1/2	1/4	1/8	1/2	1/4	1/8	1/2	1/4	1/8
Tebusan	7.5	4.0	4.5	6.5	4.0	6.0	7.4	4.7	6.8
Garlon	100	100	12.7	100	100	11.0	100	100	11.5
DCMU	82.0	78.0	44.3	79.0	74.0	48.0	80.0	72.0	66.1
No herbicide	0	0	0	0	0	0	0	0	0
LSD 0.05	2.1	4.0	6.4	3.5	11.6	4.8	2.5	2.7	4.9

3.1.3 Trial 3 (Beau Champ)

3.1.3.1 Effect of herbicide treatments on incidence of foliar interveinal chlorosis.

Interveinal foliar chlorosis of lower leaves was observed with treatments DCMU, Tebusan, and Tebusan + DCMU at 4 weeks after planting. The incidence was significantly higher with Tebusan + DCMU and DCMU while differences in % incidence among Tebusan, DMA6 and Atrazine were not significant (Figure 1). However, all treatments showed the characteristic foliar chlorosis by harvest. This suggests that the carryover of herbicides from sugar cane to tobacco lasted longer than nine months, especially for Tebusan and DCMU, where their persistence in the soil as well as their byproducts ranged between 6 to 12 months (WSSA, 1994).

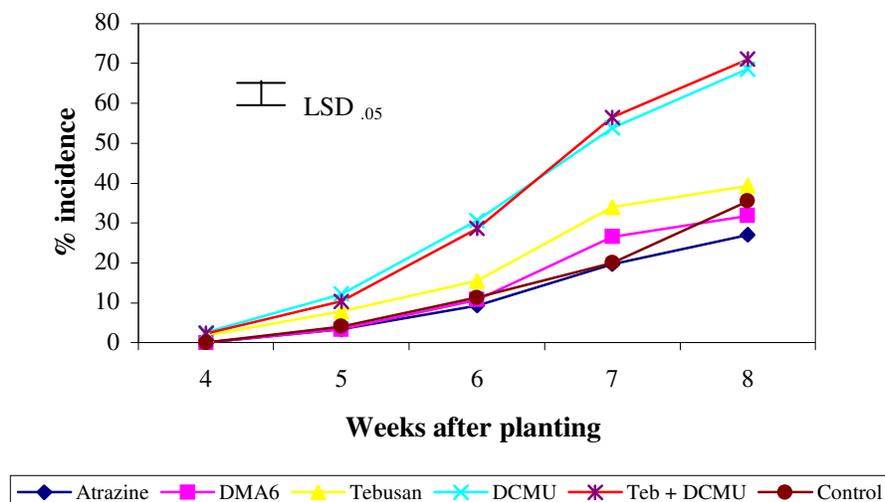


Figure 1: Effect of different herbicide treatments on incidence of interveinal yellowing in flue-cured tobacco variety RG13 at Beau Champ before harvest.

3.1.3.2 Multi residue herbicide analysis

Tebuthiuron was found present in both soil and leaf samples, while the levels of Diuron and Trichlopyr were below the quantifiable limit (Table 6). This might be ascribed to the fact that the latter two herbicides were applied as spot applications along roadsides and bedrock exposures.

Table 6: Amount of herbicide residues in tobacco leaf and in different soil horizons.

Active ingredient	Herbicide Residue (mg kg^{-1})				
	Leaf		Soil		
	Sample 1	Sample 2	0-10 cm	10- 20 cm	20- 30 cm
Diuron	< QL	< QL	< QL	QL	<QL
Tebuthiuron	0.77	0.58	0.72	0.67	0.25
Trichlopyr	< QL	< QL	< QL	< QL	< QL

QL: Quantifiable limit = 0.02 mg kg^{-1}

The results thus lend supportive evidence to the simulation trials that Tebusan is among one of the herbicides responsible for leaf disorder in flue-cured tobacco.

In all three trials, symptoms of interveinal chlorosis were apparent around 35- 40 days after transplantation, which coincided with a period of rapid root development. At this stage, the roots are extensive enough to take-up nutrients for plant growth. Photosynthetic inhibitor herbicides (triazinones, phenyl ureas, uracils and phenyl- pyridines) are taken up into the plant via the roots, move through the xylem into plant leaves, and cause injury symptoms such as interveinal yellowing of leaf tissue, followed by necrosis. Since the lower leaves were affected first, and that the symptoms observed resembled those of photosynthetic inhibitor herbicides, and given that Tebusan, Garlon, Karmex Flo, DCMU and Velpar belong to the family of photosynthetic inhibitors, the cause of the disorder could likely be attributed to uptake of these residual herbicides via the tobacco roots. Similar results were reported for tobacco and cucumber where fluometuron and other substituted urea herbicides caused interveinal chlorosis on lower leaves (Rogers et al; 1986, Bradley et al, 2001).

3.1.3.3 Effect on growth, yield and quality of tobacco

At Beau Champ, carry-over of the different herbicide treatments resulted in significantly reduced flowering interval, leaf number/plant and plant height compared to the control (Table 7). Generally, these herbicides block photosynthesis by binding to specific sites, thereby causing stunting of the growing point (Gonsolus and Curran, 1996).

Table 7: Carryover effects of herbicides used alone or in combination on tobacco growth at B. Champ in 2006.

Treatment	Days to 50% flowering	Leaf number/plant	Plant height at topping (cm)
Control (No herbicide)	48.3 ^c	15.9 ^b	75.2 ^b
Atrazine	50.3 ^b	16.9 ^b	73.8 ^b
DMA6	48.3 ^c	16.4 ^b	76.5 ^b
Tebusan	48.3 ^c	15.4 ^b	72.7 ^b
DCMU	47.7 ^c	15.6 ^b	72.8 ^b
Tebusan + DCMU	47.3 ^c	15.3 ^b	74.5 ^b
Healthy crop	59.7 ^a	20.1 ^a	88.4 ^a
SE±	0.46	0.67	3.01

Means followed by a common letter are not significantly different at the 5% level of confidence by the DMRT.

Table 8: Yield and quality comparisons at Beau Champ (affected crop)

and Ernest Florent (healthy crop) in 2006 (Mean of 3 replicates).

Site	Green leaf yield (kg ha ⁻¹)	Cured leaf yield (kg ha ⁻¹)	Grade index (Rs kg ⁻¹)
Beau Champ	6429 ^b	857 ^b	107.7 ^a
Ernest Florent	12174 ^a	1593 ^a	117.2 ^a
SE±	798.3	134.8	9.17

Means followed by a common letter are not significantly different at the 5% level of confidence by the LSD Test.

Under the given set of conditions at Beau Champ, where leaf disorder incidence ranged between 15- 60 %, both green and cured leaf yields decreased significantly by 46 %. Differences in grade index, although not significantly significant, decreased by Rs 10 kg⁻¹ for the Beau Champ location compared to Ernest Florent (Table 8). This decrease represents a loss in revenue of Rs 18,000 ha⁻¹, which could have been recouped to cater for land preparation and transplantation of tobacco.

4. CONCLUSION AND RECOMMENDATIONS

All three herbicide simulation trials reproduced symptoms similar to those observed in tobacco fields in the Beau Champ factory area. Such symptoms were attributed to carryover of herbicides Tebusan, Garlon, Karmex Flo, DCMU and Velpar. Growers should verify the previous history of fields destined for tobacco cultivation, especially in relation to any recent herbicide application. As a precautionary measure, fields which have been sprayed with herbicides in the phenylurea, pyridine and triazinone families, or any other similar chemical, during the last or two-last sugar cane cropping cycles should be avoided. Furthermore, in the light of the recent observations made at Richelieu CRS regarding the susceptibility of the tobacco varieties to the herbicides, such recommendations should be extended to all varieties, irrespective of any prior indication of tolerance and susceptibility.

5. ACKNOWLEDGEMENTS

The authors are grateful to tobacco growers and the Beau Champ Sugar Estate for their help in the management of field trials. Thanks are expressed to the Tobacco Board and the Agricultural Research and Extension Unit for funding the project.

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