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The Effect of Shade on Leaf Characteristics of *Mikania micrantha* (Compositae) and Their Influence on Retention of Imazapyr

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ABSTRAK

Lindungan telah mengakibatkan perubahan terhadap ciri-ciri histologi daun Mikania micrantha *H.B.K., dimana daun yang berada pada intensiti cahaya yang tinggi adalah lebih tebal daripada daun dari intensiti cahaya yang rendah. Peningkatan dengan kadar yang bermakna bagi luas semburan dan retensi imazapyr di permukaan atas daun dalam intensiti cahaya yang rendah menunjukan lindungan telah menukarkan topografi permukaan atas dan kuantiti lilin daun.*

ABSTRACT

Shading led to changes in the leaf histological characteristics of Mikania micrantha *H.B.K., leaves at higher light intensity being thicker than those at lower light intensity. There was a significant increase in the area of spread of imazapyr droplets and retention on the upper leaf surface at lower light intensity, suggesting that shading had changed the upper surface topography and the amount of epicuticular wax of the leaves.*

INTRODUCTION

Mikania micrantha H.B.K. is a pernicious weed in crops such as rubber, cacao, oil palm, coconut, banana, pepper and tea. It usually grows profusely in places receiving high rainfall or in humid habitats (Holm *et al.* 1977). The obnoxious character of this weed is mainly due to its rapid growth and spread, which smothers neighbouring plants, as well as its ability to root at the nodes when the stem comes in contact with soil (Macalpine 1959). Ipor (1991) reported that *M. micrantha* is a shade-tolerant species and persists in sites receiving 25% of full sunlight.

Imazapyr (isoproypylamine salt of 2-(4-isopropyl-4-methy1-5-oxo-2-imidazoin-2-yl) nicotic acid) is a broad spectrum herbicide commonly used for controlling both annual and perennial weeds (Fine *et al.* 1983). It is a systemic herbicide and is readily absorbed and translocated in plant tissues (Mallipudi *et al.* 1986). The uptake, translocation and activity of imazapyr increases significantly as light intensity decreases (Ipor and Price 1990).

It is well known that level of light intensities during growth can markedly alter the morphological, anatomical, physiological and biochemical properties of leaves. Ipor (1989) found that growth pattern, rate of expansion, final leaf area and specific area of individual leaves of *M. micrantha* were greatly influenced by shade or light intensities.

Daubenmire (1970) reported that epicuticular wax and distribution of crystalline wax had a significant effect on herbicide penetration. Hence, the objective of this study was to determine the role of light intensity in altering the topographical characteristics of wax, epidermal cell size and morphological structure of *M. micrantha*, which are likely to influence the retention of imazapyr.

MATERIALS AND METHODS

Midrib Sectioning for Light Microscopy

Plants were grown by using the procedures described by Price and Ipor (1990). The middle part of the second youngest lamina of

M. micrantha of plants grown under three levels of shade (0, 50 and 75%) was cut into a piece of 7 x 7 mm and fixed in formalin-acetic acid (FAA) under reduced pressure for a week. The samples were dehydrated in a graded ethyl alcohol series. After dehydration, the tissue was infiltrated and embedded in paraffin, which allowed to solidify. Sections 10 μm thick were stained with alcian blue and safranin (50% alcohol) (Sass 1958).

For stomata counting, the middle portion of the preserved leaves was cut into pieces 10 x 10 mm. The tissues were thoroughly washed with distilled water then gently boiled in 45 ml of 15% nitric acid until the adaxial and abaxial surfaces separated. The tissues were gently transferred into a petri dish and washed with two changes of distilled water. The remaining mesophyll was brushed away with a fine brush. The cuticles were then treated with 5% acetic acid for 30 sec and immediately transferred into sodium hypochlorite (NaOCl) for 30 min until the tissues became clear. They were then washed twice with distilled water before being transferred into 50% ethanol for 2 min followed by staining with 50% safranin for 10 min. After dehydration, the cuticles were mounted in Canada balsam or euparal and the slides dried on a hot plate for two weeks. The stomata were counted under a light microscope.

Scanning Electron Microscopy of Upper Leaf Surface

Leaf pieces 0.75 x 0.75 cm were taken from the middle portion (avoiding the midrib) of the second youngest leaf of plants grown at the three levels of shade mentioned above as well as from plants growing naturally in an open habitat in an oil palm plantation in Malaysia. The leaf pieces were affixed to aluminium stubs with colloidal silver adhesive and immediately cooled in liquid nitrogen. Specimens were then freeze dried between at -40 – -60°C for 48 h.

Specimens were coated with gold and examined and photographed using a Jeol T20 scanning electron microscope (SEM) at 3200x magnification.

Wax Deposits on the Leaf Surface

The amount of epicuticular wax was estimated gravimetrically in a chloroform extract, using a method similar to that of Souza and Williams (1986). Fully expanded leaves from the three shade regimes were excised and their surface areas determined using a photomax tracer. The leaves were then dipped in chloroform twice for 10 sec, which was then filtered through Whatman No. 1 paper into a pre-weighed test tube. Test tubes with extracts were placed in a fume hood to evaporate the chloroform and then in a forced air oven at 45°C before being transferred into a vacuum desiccator and dried to constant weight.

Droplet Spread on Leaf Surface

A Buckard microapplicator was used to apply 0.2 μl droplets of lisamine red formulations on the upper leaf surfaces (Mabb and Price 1986). The eighth and second youngest leaves of plants at the ten-leaf growth stage were used. The diameter of the droplet deposit was measured after 24 hours with a calibrated graticule eyepiece and the area calculated. The experiment was carried out with thirty replicates and repeated twice.

Spray Retention of Imazapyr

Fifty-leaf stage plants were used. The plants were sprayed with solutions containing the soluble dye lisamine (1% w/v) with imazapyr (0.3 kg a.i/ha) and distilled water. A maldrive spraying system was used to deliver 211 1/ha.

After the sprayed deposits had dried, the leaves were detached from each plant and the sprayed deposits were washed off with 25 ml of distilled water. The lisamine concentration was measured in a spectrophotometer at wavelength 460 μm. The value of the peak point was compared with a standard concentration curve for the calculation of the equivalent amount of herbicide in the sprayed deposit. Data were expressed in μg herbicide per cm^2 of leaf. The experiment was repeated twice with thirty replicates.

RESULTS

Histology of M. micrantha

The effect of shading on the leaf characteristics and anatomy are presented in *Plate 1* and Table 1. Leaves grown at 0% shade in both glasshouse and the open area in the field were significantly thicker than those at other light regimes. The number of cells between the upper and lower leaf surfaces increased with increase in light intensity.

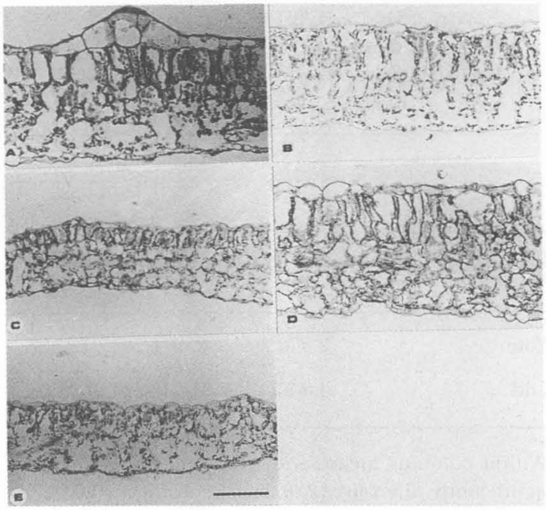

Plate 1: Transverse sections of Mikania micrantha *leaves grown under (A) 0%; (B) 50%; (C) 75% shade; (D) open areas; (E) in mature oil palm plantation. (Scale bar = 60mm)*

The size of epidermal cell of leaves grown under low light intensity increased appreciably. Palisade cell diameter also increased significantly with light intensity.

Upper Leaf Surface of M. micrantha

The width of epidermal cells on the upper surface decreased appreciably with increasing light intensity (*Plate 2*). The epidermal cells of leaves from 0% shade in the glasshouse and open areas in the field were fewer than of leaves from 75% shade (Table 1). The wax on the upper leaf surface was generally flattened.

Wax Deposits on Leaf Surfaces

The average amount of chloroform-soluble wax from leaves under different shade levels is presented in Table 2. The quantity of epicuticular wax per unit area of leaves increased as the level of shade decreased: the amount from leaves under 0% shade was more than twice that from 75% shade.

Droplet Spread on Upper Leaf Surface

The area of spread of droplets on both young and old leaves at 75% shade was significantly greater than that at 0 and 50% shade (Table 3),

TABLE 1

Effect of shading on the histological characteristics of leaves of *Mikania micrantha*

	Glasshouse			Field	
Leaf cell characters	0%	50%	75%	Open	Shaded
Number of stomata per mm^2					
Upper surface	142a	73c	10d	101b	12d
Lower surface	597a	381b	295c	298c	234d
Leaf thickness (μm)	207a	194b	136c	210a	105d
Number of cells from upper to lower leaf surface	7.8a	6.1bc	5.5cd	6.4b	4.8d
Average width of:					
Epidermal cell (μm)	32.6b	38.5a	42.7a	28.1b	22.5c
Xylem (μm)	13.4b	20.9a	18.9a	19.8b	13.3b
Phloem (x 10^{-3} μm)	7.0a	8.5a	9.1a	7.7a	8.8a
Mesophyll cell (μm)	25.2b	32.8a	25.4b	31.1a	23.3b
Palisade cell (μm)	60.6a	54.4b	40.0d	46.3c	25.6e

Within each row, values sharing the same letter are not significantly different at 5% level, according to Duncan's multiple range test.

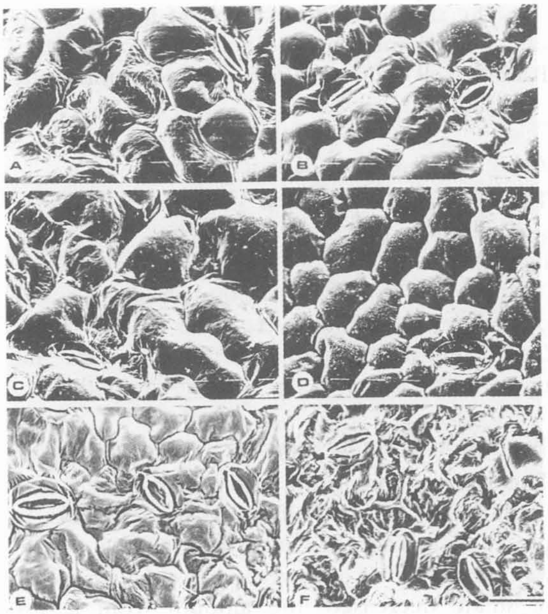

Plate 2: EM micrographs showing leaf surface of Mikania micrantha *at different levels of shade (A) 0%; (B) 50%; (C) 75% shade; (D) open areas; (E) in mature oil palm plantation: (F) without epicuticular wax after stripping with cellulose acetate. (Magnification = 3200x, Scale bar = 25cm)*

TABLE 2
Effect of shading on the quantity of epicuticular wax deposits on the leaf surface of *Mikania micrantha*

Shade Level	Mean Weight of Epicuticular Wax (μm cm^{-2})
0%	8.2a
50%	5.2b
75%	3.4a

Within each column, values sharing the same letter are not significantly different according to Duncan's multiple range test.

but there was no significant difference between 0 and 50% shade on young leaves.

Spray Retention of Imazapyr on M. micrantha *Leaves*

There was a trend towards increased retention of imazapyr with shade (Table 4). Plants under 75% shade retained more than three times that from 0% shade (Table 4).

TABLE 3
Effect of shade levels on area of spread of imazapyr drops on the upper surface of *Mikania mircrantha* leaves

	Shade level		
	0%	50%	75%
Leaf stage	Area of droplet spread (mm^2)		
Young	2.87d	2.43d	12.52c
Old	4.44d	31.01b	62.50a

Within columns means with the same letter are not significantly different (P>0.05) according to DMRT

TABLE 4
Amount of imazapyr retained on leaves of *Mikania micrantha* grown under different shade levels

Shade level (%)	Imazapyr spray retained (μg cm^{-2})*
0	0.95b
50	1.17b
75	3.16a

*Means with the same letter within the column are not significantly different (P>0.05) according to DMRT

DISCUSSION

Plants grown under high light intensities showed differences in leaf surface structure from those grown at low light intensities (Table 1). High light intensity reduces leaf expansion resulting in thicker leaves. Boardman (1977) found that leaves of *Atriplex patula* grown at 20 mw cm^{-2} were seven cells thick compared with three or four cells in leaves grown at 2 mw cm^{-2}. The mesophyll cells grown under low light intensity were smaller and more densely packed and there were fewer vascular strands. The size of the epidermal cell of *M. micrantha* was considerably smaller under high light intensity.

Shade was observed to play an important role in the development of the epicuticular waxes of *M. micrantha*. A greater deposit of epicuticular wax was found under higher light intensities

(Table 2). Skoss (1955) found that shaded leaves of ivy (*Hedera helix*) had less cuticle and wax than those exposed to full sunlight. Martin and Juniper (1970) also reported that wax production on soybean (*Glycine max*) leaves increased with light intensity. Significant increase in wax of field bindweed (*Convolvulus arvensis*) leaves was also reported under high light intensities (Steward *et al.* 1986).

A significant increase in the droplet spread was observed on leaves from shaded plants (Table 3). Dorschner and Buchholtz (1956) found that shading by companion crops increased the wettability of alfalfa (*Medicago sativa*) leaves. In the present study, wettability was significantly correlated with differences in quantity and density of the epicuticular wax crystals, and to the size of the epidermal cells of *M. micrantha*.

There was a trend toward greater retention of imazapyr on *M. micrantha* leaf surfaces grown under shade (Table 4). Differential spray retention is dependent on leaf surface characteristics and the angle of incident of the spray droplet to the leaf (Ennis *et al.* 1952; Brunskill 1956; Blackman 1958). Ennis *et al.* (1952) reported that the waxy layer on the leaf was an important characteristic affecting spray droplet repulsion. In this study, the waxy layer of *M. micrantha* leaves grown at high light intensities repelled spray droplets more effectively than those grown at lower light intensity. In heavily waxed leaves, Brunskill (1956) showed that spray droplets bounced off the leaf because the angle of incidence had decreased. This may be the main explanation why more imazapyr is retained on the leaf surface of *M. micrantha* grown under lower light intensity and which are less waxy. In addition *M. micrantha* at 0 and 50% shade levels had leaves which were slightly erect and facilitated runoff, whereas at 75% shade, the leaves were oriented horizontally, bigger and proportionally longer. Less bounce of droplets should occur at 75% shade, which may contribute to the greater spray retention observed at 75% shade.

Changes in morphology, surface structure and histological characteristics influence retention of imazapyr on plants exposed to different shade levels. Price and Ipor (1990) reported that leaves of *Paspalum conjugatum* grown under low light intensity (75% shade) had significantly increased uptake and translocation of imazapyr, and ascribed this to the thinner leaves with higher permeability. Uptake and translocation have repeatedly been shown to account for the effectiveness of herbicides (Jensen 1982). The greater susceptibility of plants growing under higher shade levels means that a smaller quantity of the herbicide and less frequent application of herbicide are needed.

REFERENCES

BLACKMAN, G.E. 1958. Differential spray retention and selective action of herbicides. In *Proceedings 1st African Weed Control.* Saffron Walden: British Crop Protection Conference.

BOARDMAN, N.K. 1977. Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology* **28:** 355-377.

BRUNSKILL, R.T. 1956. Physical factors affecting the retention of spray droplet on leaf surfaces. *Proceedings of the British Weed Control Conference* **3:** 593-603.

DAUBENMIRE, R. 1970. Steppe vegetation of Washington. *Bulletin of the Washington Agricultural Experimental Station* **62:** 80-82.

DORSHCHNER, K.P. and K.P. BUCHHOLTZ. 1956. Wetting ability of aqueous herbicidal sprays as a factor influencing stands of alfalfa seedlings. *Agronomy Journal* **48:** 59-63.

ENNIS, W.B., R.E. WILLIAMS and K.P. DORSCHNER. 1952. Studies on spray retention by leaves of different plants. *Weeds* **1:** 274-286.

FINE, R.R., T.R. PEOPLES and D.R. CIARLANTE. 1983. AC 252925 - A new broad spectrum herbicide. *Proceedings of the 9th Asian Pacific Weed Science Conference,* p. 436-439. Asia Pacific Weed Society.

HOLM, L.G., D.L. PLUCKNETT, J.V. PANCHO and J.P. HERBERDER. 1977. *The World's Worst Weeds: Distribution and Biology.* University Press of Hawaii.

IPOR, I.B. 1989. Action of herbicides on *Mikania micrantha* H.B.K. and *Paspalum conjugatum* Berg. PhD thesis, University of London.

IPOR, I.B. 1991. The effect of shade on the growth and development of *Mikania micrantha* H.B.K. *Malaysian Applied Biology* **20(1)**: 57-63.

IPOR, I.B. and C.E. PRICE. 1990. Effect of shading on the uptake and translocation of ^{14}C- paraquat and ^{14}C- imazapyr in *Paspalum conjugatum* Berg. In *Proceedings of 3rd Tropical Weed Science Conference.* Kuala Lumpur: Malaysian Plant Protection Society.

JENSEN, K.I.N. 1982. The roles of uptake, translocation and metabolism in the differential intraspecific responses to herbicides. In *Herbicide Resistance in Plants,* ed. H.M. Lebaron and J. Gressel, p. 133-162. New York: Wiley-Interscience.

MABB, L.P. and C.E. PRICE. 1986. Fluazifop-butyl activity of *Imperata cylindrica* (L) P. Beauv. (1) ·Studies on phytotoxicity, spray adhesion and herbicide uptake. *Weed Research* **26:** 301-305.

MACALPINE, R. 1959. A note on *Mikania scandens. Two and a Bud* **6:** 6-8.

MALLIPUDI, B.M., B.A. KNOLL, A.L. HORNG and E.J. ORLOSKI. 1986. Absorption, translocation and soil dissipation of imazapyr under field conditions. In *Proceedings of the Weed Science Society, North Tennessee.* 20th - 22nd January. Weed Science Society North Tennessee.

MARTIN, J.T. and B.E. JUNIPER. 1970. *The Cuticle of Plants.* London: Edward Arnold.

PRICE, C.E. and I.B. IPOR. 1990. The effect of shade on the activity of paraquat and imazapyr on *Paspalum conjugatum.* In *Proceedings of the 3rd Tropical Weed Science Conference,* Kuala Lumpur, 4th-6th December, 1990. Malaysian Plant Protection Society.

SASS, J.E. 1958. *Botanical Microtechnique.* 3rd edn. Ames: Iowa State University Press.

SKOSS, J.D. 1955. Structure and composition of plant cuticle in relation to environmental factors and permeability. *Botany Gazette* **117:** 55-72.

SOUZA, I.F. and J.L. WILLIAMS. 1986. Chloroform-soluble fraction from soybean [*Glycine max* (L.) Merr.] leaves following herbicide applications. *Weed Research* **26:** 181-183.

STEWARD, L.S., A.H. HARVEY and F.D. HESS. 1986. Effect of adjuvants and environment during plant development on glyphosate absorption and translocation in field bindweed (*Convolvulus arvensis*). *Weed Science* **34:** 811-816.

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