PROMETRYN TOLERANCE OF GLANDED VS. GLANDLESS ISOLINES IN SELECTED COTTON CULTIVARS

By

JOHN DAVID FOSTER, JR.

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Thesis Approved:

Don S. Murray
Thesis Adviser

James D. Ownby

Lawal M. Verhalen

Norman N. Durham
Dean of the Graduate College

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INTRODUCTION

This thesis is of a manuscript to be submitted for publication in Crop Science, a Crop Science Society of America journal publication.

Prometryn Tolerance of Glanded VS. Glandless Isolines in Selected Cotton Cultivars

ABSTRACT

Cotton (Gossypium hirsutum L.) cultivars normally have lysigenous gland as conditioned by two dominant alleles, Gl_2 and Gl_3. Glandless isogenic lines for a number of those cultivars are available which would permit a critical test for the relationship proposed between accumulation of prometryn [N,N'-bis(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine] in the lysigenous glands and tolerance to that herbicide in cotton. Experiments were conducted in a growth chamber to measure the prometryn tolerance of glanded vs. glandless 'Empire' and 'Westburn M' isolines. Two intermediately glanded Empire were also available for analysis. I:P ratios derived from leaf fluorescence measurement curves established that photosynthetic inhibition caused by the herbicide in glanded plants was of shorter duration than in glandless plants. Fluorescence of the intermediately glanded Empire isolines suggested that Gl_2 enhances tolerance more than Gl_3. Five cotton cultivars (i.e., Empire, Westburn M, 'Delcot 277', 'TH 149', and 'Stoneville 213'), were used to evaluate prometryn tolerance in glanded vs. glandless isolines grown

in the field. Visual injury ratings and lint yields support the laboratory data. The glanded isoline within each of the five cultivars had less visual injury and higher yield than the corresponding glandless isoline. Lysigenous glands enhance prometryn tolerance, and gland density is directly associated with tolerance.

Additional index words: Gossypium hirsutum L., Isogenic lines, Herbicide resistance, Triazine resistance.

Prometryn [N,N'-bis(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine] is a selective herbicide used primarily for broadleaf weed control in cotton (Gossypium hirsutum L.). Although extensively used, prometryn is considered moderately phytotoxic and, under certain environmental conditions, may cause crop injury. Abernathy et al. (1) conducted a field study to evaluate atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] and propazine [6-chloro-N,N'-bis(1-methylethyl)-1,3,5-triazine-2,4-diamine] tolerance among 48 cotton cultivars. Crop injury ranged from 8 to 80%, indicating that the ability of cotton to tolerate those two herbicides was at least partially dependent upon cultivar differences.

Whitenberg (12) and Sikka and Davis (10) reported that ^{14}C-prometryn accumulates in the lysigenous glands of cotton and is found in association with the polyphenol compounds. The accumulation of prometryn and other triazine herbicides in those glands is suspected to be a major factor in lowering the concentration of phytotoxin generally available to injure cotton plant tissues (3, 4, 12).

The density of lysigenous glands in cotton can differ. The normal glanded condition is caused by two partially dominant genes designated as Gl_2Gl_2 and Gl_3Gl_3. The glandless condition occurs when both genes are homozygous recessive, i.e., gl_2gl_2 and gl_3gl_3. In 1982 Shepherd (9) registered glandless isogenic lines on eight genetic

backgrounds, i.e., 'Deltapine 16', 'Auburn 56', 'TH 149', 'Coker 201', 'Coker 310', 'Delcot 277', 'Stoneville 213', and Pee Dee 2165. Three isogenic lines of cotton (Fig. 1) were developed in 'Empire' by Lee (6). The four genic combinations were described as the normally glanded cultivar ($\underline{Gl_2Gl_2Gl_3Gl_3}$), a glandless isoline ($\underline{gl_2gl_2gl_3gl_3}$), and two intermediate isolines ($\underline{Gl_2Gl_2gl_3gl_3}$ and $\underline{gl_2gl_2Gl_3Gl_3}$).

If prometryn tolerance is due to localization of the herbicide within lysigenous glands and not because of inherent physiological characteristics among cultivars, then glanded vs. glandless isolines within a cultivar should differ in their ability to tolerate prometryn. Field and growth chamber studies were conducted (i) to determine whether differences in prometryn tolerance exist between glanded vs. glandless isolines of five cotton cultivars and (ii) if so, to determine how tolerance of the two intermediate Empire isolines compares with their glanded and glandless counterparts.

MATERIALS AND METHODS

Sinking Leaf-Disk Method

An in vitro study was conducted using the sinking leaf-disk method for determining prometryn tolerance. A regiment of incandescent and fluorescent bulbs were used on a light table to establish a light intensity of 300 $\mu E\ m^{-2}\ s^{-1}$. Temperature was maintained at 27°C. Methods were slightly modified from Truelove et al. (11). A cork borer was used to excise 8 mm leaf disks from Empire glanded and glandless isolines. Disks were immediately transferred to 0.05 M KH_2PO_4 buffer solutions with and without prometryn. The experiment was arranged in a completely random design with eight replications. This technique did not provide reproducible results and was abandoned.

Fluorometric Analyses of Empire Grown in vitro

A preliminary experiment was conducted in a growth chamber to evaluate whether a portable plant productivity fluorometer (Model SF-10, Richard Brancker Res. Ltd., Ottowa, Canada) could detect differences in prometryn tolerance between glanded vs. glandless Empire isolines. Seed were germinated in vermiculite and planted into 4.5 kg of a Teller loam soil (a fine-loamy, mixed, thermic Udic Argiustoll). The growth chamber was maintained at 28°C with a day length of 14 h and a light intensity of 300 $\mu E\ m^{-2}\ s^{-1}$. The plants were arranged in a randomized complete

block design with one plant/pot and four replications. When plants attained the three true-leaf stage of development, 250 ml of a prometryn solution (18 mg L^{-1}) were flooded onto the soil surface raising the concentration of prometryn in the soil to 1.0 mg kg^{-1}.

Methods for detecting triazine tolerance using a fluorometer were based on procedures by Ahrens et al. (2) to adjust the light intensity and by Shaw et al. (8) for the actual leaf measurements. Prior to taking measurements, plants were allowed to adapt to a dark environment for 15 min. Fluorescence measurements were taken on the third true leaf between the second and third major vein on the upper right-hand side of the third leaf. Data were compiled by computer, and a fluorescence curve was plotted for each plant. From the curves, a ratio of the inflection point (I) to peak fluorescence (P) was used to calculate an I:P ratio which in turn was used to indicate photosynthetic inhibition. The I:P ratio of treated plants rises as the degree of photosynthetic inhibition increases. A ratio for a normal untreated cotton plant ranged from 0.6 to 0.7; the maximum I:P ratio which can occur in a treated plant is 1.0. Analyses of variance were calculated to determine if differences existed in the ratios between glanded vs. glandless isolines. Fluorometric measurements were taken at weekly intervals for 3 weeks after herbicide application.

Fluorometric analyses of glanded and glandless Empire isolines were repeated in both soil and solution. Seed were germinated in vermiculite and transplanted into either 4.5 kg of Teller loam soil or an aerated half-strength Hoagland's solution (5). Soil-grown plants were contained in 15 cm diameter pots, 10 cm high. Solution vials were 250 ml bottles wrapped with aluminum foil. Rubber stoppers with 64 mm holes through their middle were used to support the plants in solution.

Procedures for prometryn applications to soil-grown plants were identical to the initial experiment. Plants grown in solution were treated with 250 ml of half-strength Hoagland's solution containing 0.5 mg L^{-1} prometryn. Both experiments were arranged in a completely random design with one plant/pot or bottle and eight replications. Fluorometric measurements analyses were taken at weekly intervals and concluded 3 weeks following treatment. I:P ratios for treated glanded and glandless isolines were statistically analyzed and compared.

An additional experiment identical to the previously described solution experiment was conducted in a growth chamber to determine leaf fluorescence (I:P ratio) relationships among the four Empire isolines, i.e., $\underline{Gl_2Gl_2Gl_3Gl_3}$, $\underline{gl_2gl_2gl_3gl_3}$, $\underline{Gl_2Gl_2gl_3gl_3}$, and $\underline{gl_2gl_2Gl_3Gl_3}$.

Fluorometric Analyses of 'Westburn M' Grown in vitro

A prometryn tolerance experiment was conducted in a growth chamber comparing glanded vs. glandless isolines of Westburn M in a manner identical to the previous solution experiments with Empire. The experiment was repeated.

Prometryn Tolerance of Five Cultivars Grown in situ

Three field experiments were planted to determine the ability of glanded vs. glandless isolines of five cotton cultivars to tolerate prometryn. One of the experiments was lost due to insufficient rainfall and late planting. Empire, Westburn M, 'Delcot 277', 'TH 149', and 'Stoneville 213', with their glandless counterparts were planted on 21 June 1986 using a drop cone planter at Perkins, OK, in a Teller loam soil. The two intermediate Empire isolines $\underline{Gl_2Gl_2gl_3gl_3}$, and $\underline{gl_2gl_2Gl_3Gl_3}$ were also included. Plots within the experiment single rows, 9.2 m in length, and 91 cm apart. The experiment was designed in a split plot design with four replications. Due to lack of seed, only two replications were planted of the glandless Empire isoline. Prometryn was applied preemergence at 3.4 kg/ha with a compressed air, tractor-mounted sprayer.

Crop injury was visually evaluated on a plot basis as percent leaf chlorosis and necrosis. Ratings were taken at 2-week intervals for 6 weeks after herbicide application. Analyses of variance were calculated to determine if

differences existed in the ability of glanded vs. glandless isolines of the same cultivar to tolerate prometryn. Cotton was harvested on 6 Dec. 1986; lint yields were also compared among isolines within cultivars.

RESULTS AND DISCUSSION

Fluorometric Analyses of 'Empire' Grown in vitro

Data obtained from the preliminary, prometryn tolerance experiment with Empire grown in soil indicated that photosynthetic inhibition occurred in both glanded and glandless isolines (Table 1). I:P ratios in treated glanded and glandless plants were significantly greater than the controls 1 and 2 weeks following treatment. After 3 weeks, the I:P ratio for the treated glanded isoline was no longer significantly different from untreated plants. The ratio for treated glandless plants, however, remained significantly greater than untreated plants; and interveinal chlorosis and necrosis were apparent on older leaves.

When the second and larger prometryn tolerance study was conducted in soil, the results were only slightly different from the preliminary experiment. I:P ratios in treated glanded and glandless isolines again rose significantly 1 week after herbicide application (Table 2). However, 2 weeks following treatment, the ratio for the treated glanded isoline was no longer significantly different from untreated plants. Leaf fluorescence in the glandless isoline continued to increase, and interveinal chlorosis was apparent. After 3 weeks, the ratio for treated glandless plants remained significantly greater than untreated plants; and interveinal chlorosis and necrosis continued to develop.

The pattern of results for prometryn-treated Empire glanded and glandless isolines grown in solution was identical to the second large laboratory experiment with Empire grown in soil (Table 3).

Prometryn applications to four Empire isolines caused significant increases in leaf fluorescence in all plants after 1 week (Table 4). The intermediate isoline $\underline{Gl_2Gl_2gl_3gl_3}$ (Fig 1.) and the glandless isoline showed the greatest injury. After 2 weeks, the ratio for the normally glanded line was no longer significantly different than the untreated check. While the ratio for the intermediate isoline $\underline{Gl_2Gl_2gl_3gl_3}$ remained significantly greater than the untreated check, it was not statistically different than the normally glanded line. The ratio for $\underline{gl_2gl_2Gl_3Gl_3}$ and the glandless isoline increased and both had significantly greater fluorescence than the untreated check or $\underline{Gl_2Gl_2gl_3gl_3}$. By week 3, differences were no longer observed among the normally glanded line and $\underline{Gl_2Gl_2gl_3gl_3}$ when compared to the check. I:P ratios for $\underline{gl_2gl_2Gl_3Gl_3}$ and the glandless isoline remained significantly greater than the check with $\underline{gl_2gl_2Gl_3Gl_3}$ showing slightly less injury than the glandless isoline.

Fluorometric Analyses of 'Westburn M' Grown in vitro

The tolerance pattern exhibited by glanded and glandless Westburn M isolines in solution was the same as that shown

by Empire in soil or in solution (Table 5). The experiment was repeated, and the results were pooled.

Prometryn Tolerance of Five Cultivars Grown in situ

Intracultivar comparisons of visual crop injury ratings for five cotton cultivars (Table 6) indicate significantly greater prometryn phytotoxicity occurred in every case in glandless isolines rather than in glanded ones. In the paired comparisons, chlorosis and necrosis ranged from two to 15 times higher in the glandless isoline. Comparisons of injury ratings among the two intermediate Empire isolines also indicated additional significant differences in tolerance. The isolines with the greatest density of glands (Fig. 1) i.e., the normally glanded isoline $\underline{Gl_2Gl_2Gl_3Gl_3}$ and the intermediate isoline $\underline{Gl_2Gl_2gl_3gl_3}$, showed less injury from prometryn applications than did the glandless isoline or the other intermediate isoline $\underline{gl_2gl_2Gl_3Gl_3}$. The $\underline{gl_2gl_2Gl_3Gl_3}$ isoline displayed significantly less injury (ranging from 37 to 55%) than the glandless line, but the $\underline{Gl_2Gl_2gl_3gl_3}$ and $\underline{Gl_2Gl_2Gl_3Gl_3}$ isolines displayed even less. The latter two were not significantly different from each other. These results show that $\underline{Gl_3}$ significantly reduces prometryn injury, but that $\underline{Gl_2}$ reduces it a greater degree.

Lint yields for all five cultivars further indicated that the glanded isolines within a cultivar were significantly more tolerant to prometryn applications in every case than were their glandless counterparts (Table 6). The treated

glanded isoline had significantly higher yields than the treated glandless isoline in every comparison. Yields for treated glanded isolines ranged from 1.8 to 2.5 times greater than treated glandless isolines within the same cultivar. Among the four Empire isolines, yields for $\underline{Gl_2Gl_2Gl_3Gl_3}$, $\underline{Gl_2Gl_2gl_3gl_3}$, and $\underline{gl_2gl_2Gl_3Gl_3}$ were not significantly different; but all were significantly greater than the glandless isoline. These differences in yield, however, cannot all be attributed to prometryn tolerance vs. susceptibility. Glandless types of cotton are generally more susceptible to insects than are glanded types on the same genetic background (7), therefore, insect damage on glandless plants may have had an effect on yield.

From these data, it follows that the normally glanded and glandless isolines within a cultivar (i.e., within a single genetic background) have separate and distinct abilities to tolerate prometryn. When glanded and glandless isolines of the same cultivar were subjected to identical quantities of prometryn and treated identically in every other way possible, the glanded isoline was better able to withstand the resulting phytotoxicity. Photosynthetic inhibition caused by the herbicide was of shorter duration in glanded plants. Earlier investigators have reported that prometryn accumulates in the lysigenous glands. Thus, it seems probable that glanded isolines are better able to immobilize and/or isolate enough prometryn from the plant tissues to

reduce phytotoxicity. Our field and laboratory evaluations of glanded vs. glandless isolines from five cultivars support this hypothesis. Furthermore, data from the four Empire isolines when subjected to prometryn suggest tolerance is dependent on the gland density of the isoline evaluated. Those Empire isolines with the greatest density of glands were more tolerant to prometryn applications. Thus, a strong positive relationship exists between the density of glands present and tolerance. $\underline{Gl_2}$ or $\underline{Gl_3}$ by themselves will enhance tolerance, but $\underline{Gl_2}$ appears to be more effective in doing so. This is the same sort of relationship that Wilson (13) found for larval resistance to tobacco budworm [*Heliothis virescens* (F.)]. Whether selective breeding to increase the density of lysigenous glands will improve prometryn tolerance has yet to be determined.

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Table 1. Preliminary in vitro analysis of leaf fluorescence (I:P ratio) in glanded and glandless 'Empire' isolines grown in soil with and without prometryn.

Empire isoline	Prometryn rate	Weeks after treatment 1	2	3
	mg kg^{-1}	--------- I:P ratio --------		
$\underline{Gl_2Gl_2Gl_3Gl_3}$	0.0	0.71 a*	0.68 a	0.62 a
	1.0	0.82 b	0.80 b	0.67 a
$\underline{gl_2gl_2gl_3gl_3}$	0.0	0.66 a	0.69 a	0.66 a
	1.0	0.92 b	0.90 b	0.93 b

*Means within a column followed by the same letter are not significantly different at the 0.05 probability level (according to an LSD test).

Table 2. In vitro analysis of leaf fluorescence (I:P ratio) in glanded and glandless 'Empire' isolines grown in soil with and without prometryn.

Empire isoline	Prometryn rate	Weeks after treatment 1	2	3
	mg kg^{-1}	--------- I:P ratio --------		
$\underline{Gl_2Gl_2Gl_3Gl_3}$	0.0	0.68 a*	0.61 a	0.69 a
	1.0	0.84 b	0.76 a	0.67 a
$\underline{gl_2gl_2gl_3gl_3}$	0.0	0.68 a	0.65 a	0.68 a
	1.0	0.89 b	0.93 b	0.96 b

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level (according to an LSD test).

Table 3. In vitro analysis of leaf fluorescence (I:P ratio) in glanded and glandless 'Empire' isolines grown in solution with and without prometryn.

Empire isoline	Prometryn rate	Weeks after treatment 1	2	3
	mg L^{-1}	-------- I:P ratio ---------		
$\underline{Gl_2Gl_2Gl_3Gl_3}$	0.0	0.69 a*	0.72 a	0.73 a
	0.5	0.80 b	0.79 a	0.73 a
$\underline{gl_2gl_2gl_3gl_3}$	0.0	0.73 a	0.73 a	0.71 a
	0.5	0.82 b	0.87 b	0.89 b

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level (according to an LSD test).

Table 4. In vitro analysis of leaf fluorescence (I:P ratio) in glanded, intermediately glanded, and glandless 'Empire' isolines grown in solution with and without prometryn.

Empire isoline	Prometryn rate	Weeks after treatment		
		1	2	3
	mg L^{-1}	--------- I:P ratio ---------		
$Gl_2Gl_2Gl_3Gl_3$	0.0	0.70 a*	0.71 a	0.72 a
$Gl_2Gl_2Gl_3Gl_3$	0.5	0.78 b	0.74 ab	0.72 a
$Gl_2Gl_2gl_3gl_3$	0.5	0.87 c	0.78 b	0.76 a
$gl_2gl_2Gl_3Gl_3$	0.5	0.81 b	0.89 c	0.90 b
$gl_2gl_2gl_3gl_3$	0.5	0.87 c	0.92 c	0.96 c

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level (according to an LSD test).

Table 5. In vitro analyses of leaf fluorescence (I:P ratio) in glanded and glandless 'Westburn M' isolines grown in solution with and without prometryn. Results pooled over two experiments.

Westburn M isoline	Prometryn rate	Weeks after treatment 1	2	3
	mg L^{-1}	---------- I:P ----------		
$Gl_2Gl_2Gl_3Gl_3$	0	0.72 a	0.72 a	0.71 a
	0.5	0.82 b	0.78 a	0.72 a
$gl_2gl_2gl_3gl_3$	0	0.72 a	0.73 a	0.71 a
	0.5	0.86 b	0.90 b	0.94 b

*Means within a column followed by the same letter are not significantly different at the 0.05 probability level (according to an LSD test).

Table 6. Visual injury rating and lint yield of five cotton cultivars and their glandless and/or intermediately glanded isolines when treated with and without prometryn in the field.

Cultivar and isoline	Visual injury rating			Lint yield †	
	July 5	July 19	Aug. 2	Untreated	Treated
	------- % injury ------			--- kg ha^{-1} ----	
Empire					
$\underline{Gl_2Gl_2Gl_3Gl_3}$	5 a *	7 a	8 a	803 a	778 a
$\underline{Gl_2Gl_2gl_3gl_3}$	2 a	8 a	5 a	776 a	738 a
$\underline{gl_2gl_2Gl_3Gl_3}$	25 b	13 b	12 b	746 a	726 a
$\underline{gl_2gl_2gl_3gl_3}$	45 c	35 c	28 c	719 a	338 b
Westburn M					
$\underline{Gl_2Gl_2Gl_3Gl_3}$	10 a	21 a	18 a	939 a	859 a
$\underline{gl_2gl_2gl_3gl_3}$	45 b	42 b	61 b	886 a	412 b
Delcot 277					
$\underline{Gl_2Gl_2Gl_3Gl_3}$	11 a	14 a	9 a	851 a	829 a
$\underline{gl_2gl_2gl_3gl_3}$	40 b	56 b	51 b	765 a	465 b
TH 149					
$\underline{Gl_2Gl_2Gl_3Gl_3}$	8 a	14 a	11 a	866 a	899 a
$\underline{gl_2gl_2gl_3gl_3}$	44 b	42 b	40 b	778 a	470 b
Stoneville 213					
$\underline{Gl_2Gl_2Gl_3Gl_3}$	4 a	18 a	12 a	954 a	941 a
$\underline{gl_2gl_2gl_3gl_3}$	60 b	59 b	62 b	877 a	373 b

* Means within a column and within a cultivar followed by the same letter are not significantly different at the 0.05 probability level (according to an LSD test).

† Prometryn was applied at the rate of 3.4 kg ha^{-1}.

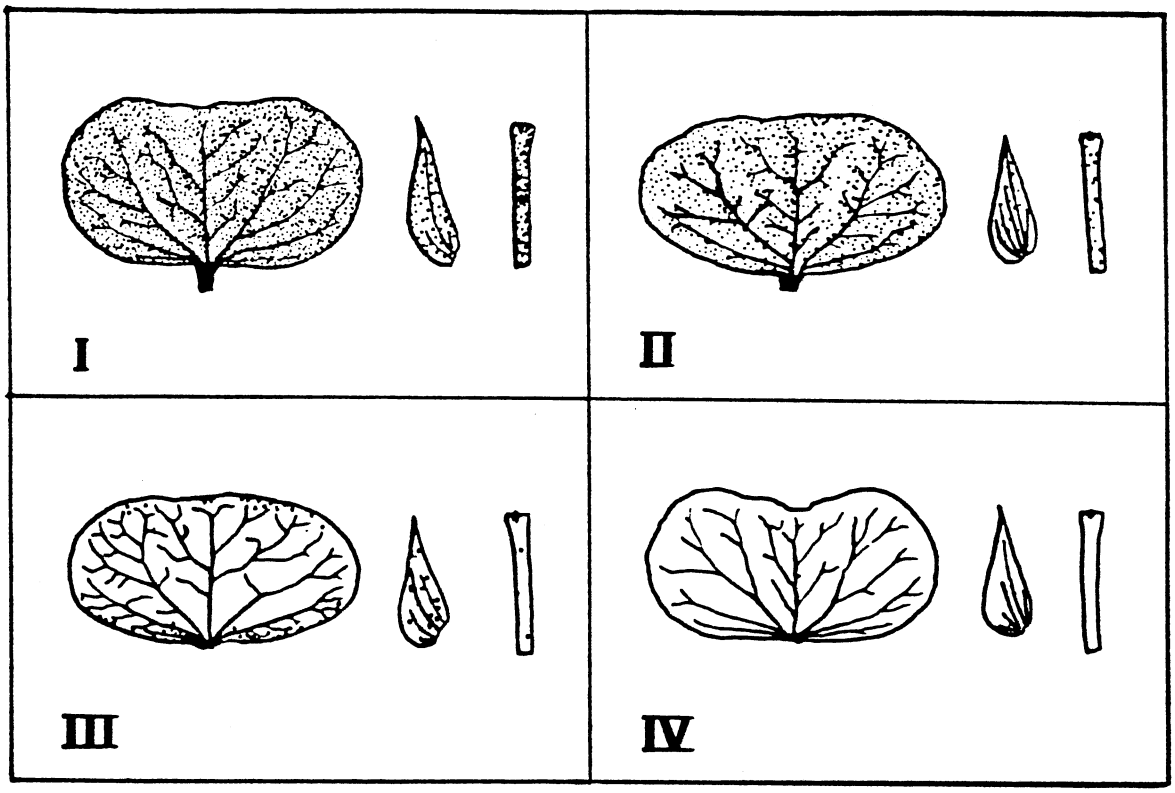

Fig. 1. Relative distribution of lysigenous glands on the cotyledons, stipules, and hypocotyls of cotton plants. I represents a normally glanded plant $\underline{Gl_2Gl_2Gl_3Gl_3}$. II and III illustrate the intermediate isolines $\underline{Gl_2Gl_2gl_3gl_3}$ and $\underline{gl_2gl_2Gl_3Gl_3}$, respectively. IV shows the glandless isoline $\underline{gl_2gl_2gl_3gl_3}$. [This illustration was redrawn from McMichael (8)].

VITA 2

JOHN DAVID FOSTER, JR.

Candidate for the Degree of

Master of Science

Thesis: PROMETRYN TOLERANCE OF GLANDED VS. GLANDLESS ISOLINES IN SELECTED COTTON CULTIVARS

Major Field: Agronomy

Biographical:

Personal Data: Born in Blackwell, Oklahoma, on December 5, 1961, the son of Dr. and Mrs. John D. Foster, Sr.

Education: Graduated from Ponca City High School, Ponca City, Oklahoma, in May 1980; received the Bachelor of Science degree in Zoology from Oklahoma State University in May 1985. Completed requirements for the Master of Science degree from Oklahoma State University in July, 1987.

Professional Experience: Research Aide, Department of Agronomy, Oklahoma State University, May 1982 to May 1985; Research Assistant, Department of Agronomy, Oklahoma State University, May 1985 to July 1987.

Professional Memberships: American Association for the Advancement of Science, Southern Weed Science Society.