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Morphological Response of Two Mesquite Varieties to 2,4,5-T and Picloram[1]

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Abstract. Honey mesquite [*Prosopis juliflora* var. *glandulosa* (Torr.) Cockerell] and velvet mesquite [*P. juliflora* var. *velutina* (Woot.) Sarg.] seedlings were treated on individual leaves with 20 or 40 μg of (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T), 4-amino-3,5,6-trichloropicolinic acid (picloram), or a 1:1 mixture thereof. Formulation of herbicides in a DMSO-complex carrier (dimethyl sulfoxide, ethylene glycol, phytobland oil, water: 50:25:15:10, v/v) enhanced activity considerably over that obtained with an aqueous carrier, the degree of enhancement being greater with 2,4,5-T than with picloram. Lack of major varietal differences in morphological or anatomical response suggests that observed varietal differences in sensitivity of field mesquite to aerial sprays are not a function of the variety itself, but are related to climatic or edaphic differences among sites which the varieties occupy.

INTRODUCTION

PICLORAM and 2,4,5-T have been used to kill a wide variety of woody plants (2, 3, 12). Although these herbicides are highly effective against numerous woody plant species, either alone or in combination with other herbicides, they are ineffective on certain other species or must be used at excessively high rates. Overcoming this lack of effective control in resistant species and lowering the dosage of herbicide (or the number of treatments) needed to control susceptible species are long-sought goals of the weed scientist.

In mesquite, the honey and velvet varieties are not equally susceptible to chemical control. Thus, the current recommendation for aerial control, based on work with velvet mesquite in Arizona (9), calls for two applications of 0.37 to 0.56 kg/ha of a low-volatile ester of 2,4,5-T, 1 or 2 years apart, whereas extensive studies with aerial sprays on honey mesquite in Texas (4) have demonstrated effective and economic control by a single application of 2,4,5-T at similar rates. One objective of the present work was to determine whether the apparently greater susceptibility of honey mesquite represents a true varietal difference, or whether the difference in response of the two varieties is a result of climatic and perhaps edaphic differences between the regions where they are found.

Preliminary experiments[3] with growth chamber and greenhouse-grown seedlings of the two varieties have indicated that velvet mesquite has a somewhat more upright growth habit than does honey mesquite, and also has longer stems, more leaves, and a greater quantity of dry matter when maintained under identical photoperiods and temperatures ranging from 27 to 38 C. At the same ages, the larger velvet mesquite seedlings are slightly more resistant to herbicides than honey mesquite seedlings. However, if grown to about the same size, by planting each variety on a different date, the response of the two varieties to herbicides is quite similar. Additional experiments[4] with seedlings of the two varieties ranging in age from 3 to 11 weeks and having from 10 to 40 leaves on their single stems, showed only a slight increase in resistance to 2,4,5-T with increasing age when approximately the central one-third of their leaves were treated. Actually, the degree of contact injury and apical epinasty, as well as subsequent stem callus and injury to basal leaves, failed to show any significant correlation with age in either variety. On the other hand, formative effect to the apical region by the third week after treatment, growth repression, final stem dieback, and the percentage of plants killed all showed significant negative correlations with plant age.

Growth chamber tests with velvet mesquite (6) have demonstrated that the addition of 50% DMSO to an aqueous carrier for the triethylamine salt of 2,4,5-T considerably enhanced overall herbicidal activity, whereas relatively little influence of DMSO was observed when it was combined with either picloram or 3,6-dichloro-*o*-anisic acid (dicamba). A carrier consisting of DMSO, glycerol, phytobland oil, and water (50:25:15:10, v/v) (DMSO-complex carrier) further increased the activity of the triethylamine salt of 2,4,5-T on velvet mesquite seedlings, but again was comparatively less effective in enhancing the activity of picloram and dicamba (11). Little, if any, enhancement was obtained with the butoxyethanol ester of 2,4,5-T. Closely analogous to this response is the finding of Mussell *et al.* (8) that (2,4-dichlorophenoxy)acetic acid (2,4-D) applied to the primary leaf of bean (*Phaseolus* spp.) induced abscission, but to a considerably lesser extent than did the *n*-octyl ester of 2,4-D. However, when applied in DMSO the abscission promoting activity of the free acid of 2,4-D was markedly increased, whereas the ester's activity was not further enhanced.

Because of the above findings, the triethylamine salt of 2,4,5-T rather than an ester was selected for the present work. An additional objective of the work was to evaluate relative morphological and anatomical responses of both honey and velvet mesquite seedlings to treatments of 2,4,5-T, picloram, and a 1:1 mixture of the two either in water or the above DMSO-complex carrier.

MATERIALS AND METHODS

Greenhouse-grown seedlings of honey and velvet mesquite 42 days old were used for the experiment. The

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[3]MORTON, H. L., S. J. SHELLHORN, and H. M. HULL. 1970. Comparison of growth and response to herbicides of velvet and honey mesquite seedlings. Weed Sci. Soc. Amer. Abstr. p 57.

[4]HULL, H. M., H. L. MORTON, R. L. GLINSKI, and J. R. WHARRIE, unpublished data, 1970.

honey mesquite seedlings ranged from 11 to 16 cm in height and had from 8 to 10 leaves per plant. Velvet mesquite seedlings ranged from 18 to 27 cm in height and had from 12 to 15 leaves per plant. Two replications of 10 plants each were selected for each treatment. The triethylamine salt of 2,4,5-T, the potassium salt of picloram, or a 1:1 mixture of the triethylamine salts of 2,4,5-T and picloram were used at a total concentration of 1000 ppmw. These herbicides were formulated either in a water carrier or the DMSO-complex carrier described above, except that ethylene glycol was substituted for glycerol in the carrier complex. Preliminary experiments had indicated that these adjuvants were equally effective. The phytobland oil was a highly refined isoparaffinic fraction having the approximate distillation range of kerosene. A nonionic surfactant blend of sorbitan monolaurate and polyoxyethylene sorbitan monolaurate, adjusted to a hydrophile-lipophile balance (HLB) of 12, was used throughout at 0.5% (v/v). Application was made by means of a micrometer-driven syringe at the rate of 20 µl per leaf (20 µg of herbicide) to the upper surface of leaves located on the central portion of the stem. Because of the considerably greater toxicity of formulations made up with the DMSO-complex carrier (11), seedlings receiving this formulation were treated on only one leaf, whereas seedlings receiving the water carrier were treated on two leaves.

Contact injury to treated leaves and epinasty of apical portions of the stem were evaluated 5 days after treatment. Effects of treatments on newly-formed leaves and the apical bud of each plant were evaluated 23 days after treatment. The quantity of callus formed by each plant stem was recorded 29 days after treatment. The degree of chlorosis and/or necrosis on leaves basipetal to the treated leaves, the relative amount of stem dieback on each plant, and the dry weights of 10 shoots and roots were all measured 33 days after treatment.

At the above time of harvest, stem segments which showed representative degrees of callus development were taken immediately above the portion of stem opposite the treated leaves. Segments at comparable levels on nontreated seedlings also were taken. All segments were vacuum infiltrated with FPA (formalin, propionic acid, 70% ethanol; 2:1:17, by volume), dehydrated in a tertiary butyl alcohol series, and embedded in 60 to 62 C paraffin. Transections 9 to 12 µ in thickness were microtomed at the callused regions of the stem segments, at or within 2 mm of a node. After mounting, all sections were stained with a six-dye schedule (10).

Results and Discussion

Specific morphological responses which occurred in the two mesquite varieties are shown in Table 1. They were measured at various intervals ranging from 5 to 33 days following treatment with the herbicide formulations indicated. Of the tabulated responses, those which are especially pertinent include the following:

Contact injury to treated leaves. When formulated in a water carrier, 2,4,5-T produced more contact injury on velvet mesquite whereas picloram in water caused greater injury to honey mesquite. In the combination treatment, 2,4,5-T and picloram offset each other to the extent that the varietal responses were almost identical. When formulated in the DMSO-complex carrier, all treatments produced such severe contact injury that any varietal differences in response were obscured.

Apical epinasty. Marked increase in epinasty was observed in both honey and velvet seedlings treated with 2,4,5-T in the DMSO-complex carrier as compared to treatments in the water carrier. The degree of epinasty caused by 2,4,5-T in a water carrier was significantly less, within varieties, than any other herbicide-carrier combination.

Chlorosis and injury to apical leaves and growing point. Severe chlorosis and injury to the apical growing point and newly formed leaves was observed on plants of both varieties receiving all herbicide treatments, except for velvet seedlings which were treated with 2,4,5-T in water.

Table 1. Responses of honey and velvet mesquite seedlings to foliage treatments of 2,4,5-T, picloram, or a 1:1 combination of 2,4,5-T and picloram in water or DMSO-complex[a] carriers.

Herbicide	Carrier	Mesquite variety	Contact injury, treated leaves[b] (5 days)	Apical epinasty[b] (5 days)	Chlorosis and injury to apical region[b] (23 days)	Stem callus[b] (29 days)	Injury to basal leaves[b] (33 days)	Stem dieback[c] (33 days)	Dry weight (33 days) Shoot	Dry weight (33 days) Root
(1000 ppmw)									(g)	(g)
2,4,5-T	water	honey	50 e[d]	15 ef	61 b	15 bc	6 d	10 abc	8 cdef	6 bcd
		velvet	68 c	2 f	36 c	9 c	16 bcd	3 bc	11 abcde	10 ab
2,4,5-T	DMSO-complex	honey	100 a	45 bcd	95 a	40 a	3 d	26 abc	8 cdef	6 bcd
		velvet	100 a	37 cde	100 a	39 a	14 cd	11 abc	12 abcd	9 abc
Picloram + 2,4,5-T	water	honey	81 b	62 abc	82 a	6 c	48 a	25 abc	5 f	4 c
		velvet	79 b	65 abc	86 a	7 c	28 abcd	12 abc	7 def	7 abcd
Picloram + 2,4,5-T	DMSO-complex	honey	100 a	58 abcd	90 a	20 abc	10 cd	13 abc	10 bcdef	7 abcd
		velvet	100 a	31 de	95 a	32 ab	20 bcd	9 abc	16 a	10 ab
Picloram	water	honey	81 b	72 ab	100 a	5 c	48 a	38 a	6 ef	5 cd
		velvet	58 d	80 a	81 ab	1 c	19 bcd	32 abc	8 cdef	10 ab
Picloram	DMSO-complex	honey	100 a	66 ab	98 a	1 c	25 abcd	36 ab	6 ef	5 cd
		velvet	100 a	49 bcd	81 ab	4 c	33 abc	2 bc	14 ab	11 a
Check	—	honey	0 f	0 f	0 d	0 c	42 ab	0 c	15 ab	10 ab
		velvet	0 f	0 f	0 d	0 c	34 abc	0 c	13 abc	9 abc

[a]DMSO-complex carrier consists of dimethyl sulfoxide, 50%; ethylene glycol, 25%; phytobland oil, 15%; and water, 10%.
[b]Relative responses on a percentage basis, 0 being no effect and 100 being maximum effect or death.
[c]Stem dieback as a percentage of total stem length.
[d]Mean values within each column having a common letter designation do not differ significantly at the 0.05 level of confidence, according to Duncan's Multiple Range Test.

Stem callus. The 2,4,5-T + picloram mixture and 2,4,5-T alone, when applied in the DMSO-complex carrier, caused more callus formation than did picloram alone. This response may be due to picloram's rapid toxic action, which completely killed considerable stem tissue before callus could form. Except for the picloram only treatments, stem callus on both varieties was far more intense with the DMSO-complex carrier than with the water carrier.

Injury to basal leaves. As with contact injury to treated leaves, 2,4,5-T in a water carrier caused more injury to the basal leaves of velvet mesquite while picloram in water resulted in greater injury to the basal leaves of honey mesquite. The herbicides in combination again offset each other to the extent that responsive differences between varieties were minimized. When herbicides were formulated in the DMSO-complex carrier, injury to the basal leaves was variable and varietal tendencies were not evident. It may be noted that basal leaves of the control plants actually showed more chlorosis or other damage than basal leaves of many of the treated plants. The basal leaves of older untreated mesquite seedlings often die from senescence, which may be brought about by shading or other factors. Previous experiments have demonstrated that low concentrations of 2,4,5-T actually reduce the onset of senescence in the basal leaves. This is true even if the basal leaves themselves are treated with the herbicide, providing the concentration is sufficiently low (i.e., < 300 ppmw). The phenomenon apparently is due to repression of apical meristematic activity and consequent decreased demand for metabolites from the older tissues.

Stem dieback. None of the treatments killed a great quantity of stem tissue in either variety. In all instances, individual treatments caused slightly more stem dieback in honey mesquite than in velvet mesquite. These differences could have reflected simply the somewhat smaller size of honey as compared to velvet mesquite at the time of treatment.

Effect on shoot and root dry weight. All herbicidal combinations reduced both shoot and root dry weight of honey mesquite seedlings considerably more than that of velvet mesquite. Greatest reductions to shoots and roots of honey mesquite resulted from the 2,4,5-T + picloram treatments in a water carrier. When this herbicidal mixture was formulated in the DMSO-complex carrier, it did not induce significant changes in the shoot or root dry weights of either variety, as compared to the nontreated checks. It should be recalled, of course, that the herbicidal rate per plant with the DMSO-complex carrier was only half of that with the aqueous carrier.

Stem transections of control and treated seedlings of honey and velvet mesquite are shown in Figure 1. Vascular cambium in untreated seedlings of both varieties (Parts 1 and 2) was quite distinct and was arranged in radial columns. In the majority of seedlings treated with either herbicide, increased meristematic activity in localized regions resulted in additional tiers of cambial cells and a general loss of such cambial distinction. Increased cambial activity of this nature has been described in Red Kidney bean *(Phaseolus vulgaris* L.) by Fisher *et al.* (5) following foliar treatment with picloram.

Ray and phloem parenchyma showed considerable proliferation with all treatments. Such proliferation sometimes encompassed the entire periphery of the stele (e.g., Part 5, Figure 1), but often was associated principally with adventitious root initials (e.g., "a" in Part 4, Figure 1). The induction of root initials in this manner, following treatment with 2,4,5-T, perhaps was first observed in bean by Beal (1). It is principally within this tissue and its derivatives that the root primordia are initiated. Primordia in various stages of development are evident in the micrographs of all treated seedlings with the exception of Parts 3 and 5, Figure 1. The distortion of tissue at "a" in Part 7, Figure 1, is actually the peripheral tissue of a root initial. Serial sections a short distance away disclosed a moderately advanced primordium, quite similar to that in Part 10, Figure 1. Part 8, Figure 1 shows a sector of the stem between two developing primordia, and between which the inner cortex has proliferated to an excessive thickness of about 1 mm, approximately 10 times the thickness of the cortical layer in an untreated seedling of the same age. In contrast to the above-mentioned work of Fisher *et al.* (5), where picloram induced enlarged epidermal and hypodermal cells in bean but did not influence cortical cells, we observed a marked induction of cortical proliferation with both 2,4,5-T and picloram but little or no effect on the dermal cells other than a secondary rupturing.

The xylem of all treated seedlings, particularly those receiving 2,4,5-T, showed an abnormal tier of cells, often about 20 to 25 layers deep. The xylem parenchyma and vessels within this zone matured without enlarging to a normal size. The average radial dimension of parenchyma cells, for example, was about 8 to 9 μ compared to 14 or 15 μ in the stems of untreated seedlings. This abnormal xylem is evident in Part 7, Figure 1, but was observed also following treatment with the other formulations. Meyer (7) noted a similar response in honey mesquite seedlings following the application of picloram or 2,4,5-T. In contrast to this stunting effect on xylem cell size, which generally occurred in sectors of symmetrical radial development, the excessive proliferation associated with development of adventitious root initials often resulted in xylem parenchyma cells reaching radial dimensions of 22 to 24 μ.

All treated plants showed increased quantities of starch granules in parenchyma tissue, particularly that of the pith and xylem. Starch is indicated on all of the picloram-treated plants (Parts 3 through 6, Figure 1), but occurred with an equal abundance in the 2,4,5-T-treated plants. A similar abundancy of starch was found by Meyer (7). Even though starch largely disappeared by the eighth and fifteenth days following treatment, he found it abundant again by the fifty-third day.

Although herbicides in the DMSO-complex carrier produced a far greater contact injury to the treated leaves and a generally greater quantity of stem callus than did similar formulations in a water carrier, consistent anatomical differences in the stems due to carrier were difficult to establish. The photomicrographs shown may suggest certain differences in this respect, but examination of numerous additional sections from this and other experiments disclosed variations sufficient to make inadvisable the assignment of any effect due to carrier. In contrast to this inconsistent effect of carrier on stem anat-

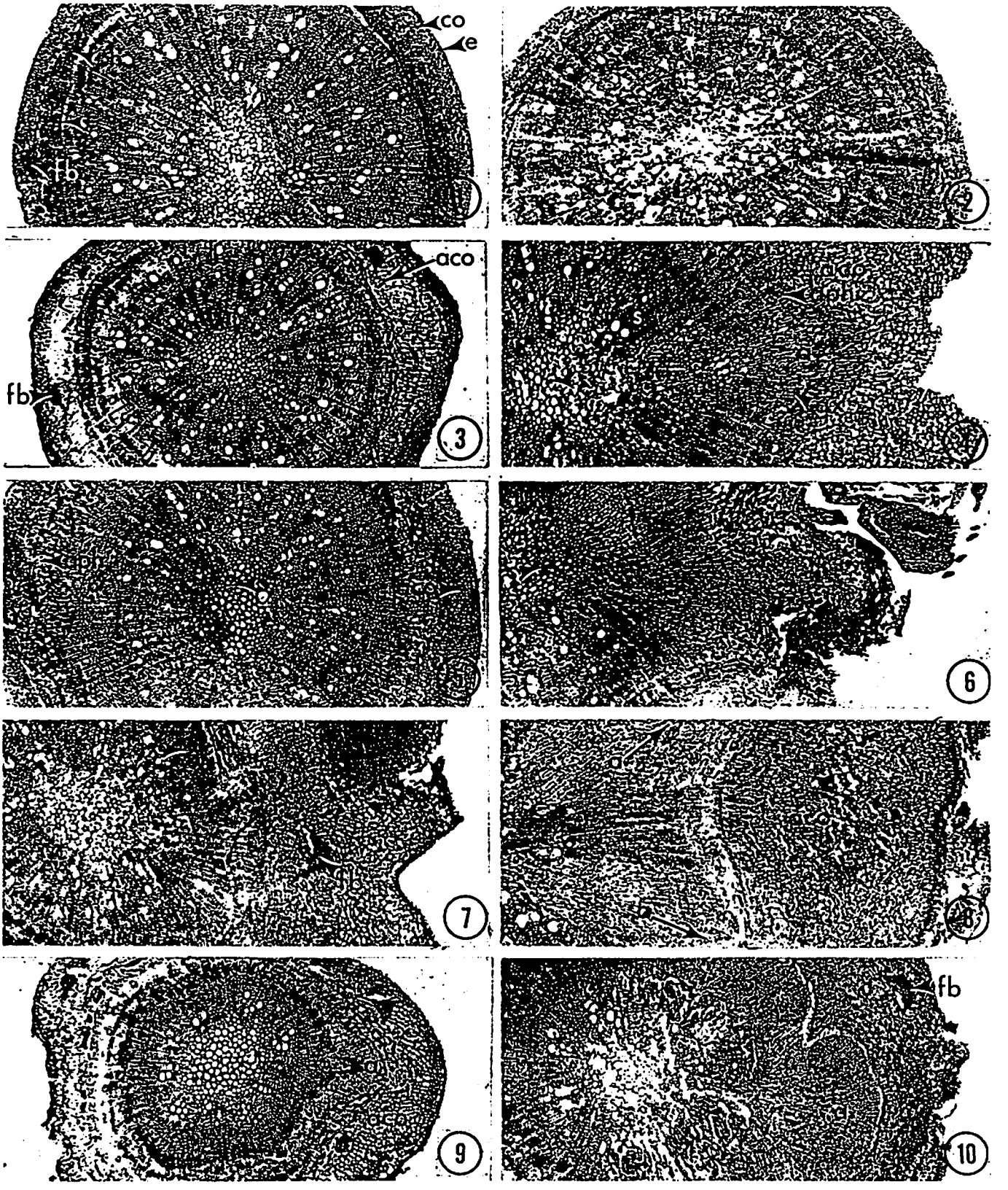

Figure 1. Stem transections of untreated and picloram or 2,4,5-T-treated greenhouse seedlings of honey mesquite *(Parts* 1, 3, 5, 7, 9) and velvet mesquite *(Parts* 2, 4, 6, 8, 10) 33 days after treatment (all X34). *Parts* 1 and 2, untreated controls. *Parts* 3 and 4, seedlings treated with 1000 ppmw picloram in a water carrier. *Parts* 5 and 6, seedlings treated with 1000 ppmw picloram in a DMSO-complex carrier. *Parts* 7 and 8, seedlings treated with 1000 ppmw 2,4,5-T in a water carrier. *Parts* 9 and 10, seedlings treated with 1000 ppmw 2,4,5-T in a DMSO-complex carrier. Details: *a*, adventitious root initial; *aco*, abnormal cortex; *aph*, abnormal phloem; *ax*, abnormal xylem; *c*, cambium; *co*, cortex; *e*, epidermis; *fb*, fibers; *ph*, phloem; *s*, starch grains; *x*, xylem.

omy, earlier work (6) had shown that the inclusion of DMSO in a carrier for 2,4,5-T induced a distinctive type of translocation injury to leaves of velvet mesquite seedlings located immediately above the treated leaves. Such injury was characterized by chloroplast destruction and convolution or even dissolution of the leaf parenchyma cell walls

Although certain quantitative differences in response of the two mesquite varieties to the herbicides also were evident, these differences for the most part were nonsignificant. Quantitative differences in response, evident principally in dieback and repression of shoot and root dry weight, could be due partially to the slight difference in size of the two varieties at time of treatment.

The ability of the DMSO-complex carrier to enhance certain morphological responses, including apical epinasty, stem callus, and subsequent chlorosis or injury to the apical region of the plant, was considerably greater with 2,4,5-T than it was with picloram, which corroborates earlier data (11). Actually, in the present work, no significant effect on picloram activity was evident, except for increased contact injury and inhibited repression of velvet mesquite shoot dry weight.

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