

INTERACTIONS OF HERBICIDE AND  
FE ON GROWTH, MINERAL  
COMPOSITION AND PHYSIOLOGY OF  
SOUR LIME AND SOUR ORANGE  
SEEDLINGS<sup>1</sup>

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ABSTRACT

One-year-old seedlings of sour lime [*Citrus aurantifolia* (Christ.) Swingle.] and sour orange (*C. aurantium*) were transplanted into plastic pots containing 10 kg virgin calcareous soil. After establishment, the plants were treated with 0, 4, and 8 kg/ha bromacil (5-bromo-3-sec-butyl-6-methyluracil) or 0, 3, and 6 kg/ha diuron [3-(3,4-dichlorophenyl)-1,1-dimethyl-urea] concurrent with 0, 5, and 10 ppm Fe as FeSO<sub>4</sub> (inorganic) or FeEDDHA (organic).

The results indicated that both FeSO<sub>4</sub> and FeEDDHA were effective if applied together with the herbicides to the soil. The interactions of bromacil and diuron with FeSO<sub>4</sub> or FeEDDHA were greatly dependent on the concentration of the herbicide and source of Fe. In general, both herbicides were

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خلاصه

نهالهای یکساله لیموترش و نارنج در گلدانهای پلاستیکی حاوی ۱۰ کیلوگرم خاک بکر آهکی کاشته شد. پس از جابجایی و رشد کافی، دو علفکش بروماسیل بمقدار صفر، ۴ و ۸ کیلوگرم در هکتار رودا یی—وران بمیزان صفر، ۳ و ۶ کیلوگرم در هکتار همراه با صفر، ۵ و ۱۰ قسمت در میلیون آهن بصورت معدنی و آلی به خاک اضافه گردید. نتایج بدست آمده نشان داد که اگر فرمهای معدنی و آلی آهن با علفکشهای بروماسیل و دایوران بکار روند نمیتوانند موثر واقع شوند. اثر متقابل بروماسیل و دایوران با آهن بصورتهای معدنی و آلی شدیداً بستگی به غلظت علفکش و منبع تا مین آهن داشت. بطور کلی، هر دو علفکش در غلظتهای بالاتر آهن و بدون توجه به منبع آهن سمیت بیشتری داشتند. ضمناً نهالهایی که بخاک آهن دو علفکش همراه با آهن اضافه شده بودند در مقایسه با شاهد حاوی مقدار بیشتری از نیتروژن و کلروفیل بودند. بطور کلی نتیجه گیری شد که اشکال معدنی آهن را که بتنهائی نسبتاً "مؤثر" نیستند میتوان همراه با علفکشها جهت برطرف کردن کلروز آهن و تسریع اثر علفکش بکار برد.

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more phytotoxic in the presence of higher than the lower levels of Fe from either source. Bromacil-Fe or diuron-Fe treated plants had higher chlorophyll and N contents than controls. It was concluded that the usually ineffective inorganic Fe could be used in combination with herbicides to effectively overcome iron chlorosis and to enhance herbicidal actions.

## INTRODUCTION

Chemical weed control, a relatively new development in Iran's citrus culture, is achieved by the use of such selective preemergence herbicides as bromacil (Hyvar X) and diuron (Karmex). In southern Iran, iron deficiency symptoms can frequently be observed in citrus orchards. It has been shown that herbicides, in addition to controlling weeds, interact with nutrients and change their absorption, translocation, and accumulation by the plants (6, 8, 9).

Selman and Upchurch (9) using diuron, observed herbicide-phosphorus interaction in grass and broad-leaved species. Burger and Vincent (3) found that treatment of citrus trees with bromacil resulted in lower yields but increased the N content of the trees. Cassin and Lossois (4) observed better N and K nutrition after two years of experimentation in an orange (*C. sinensis* Osbeck) orchard with bromacil and diuron.

Khubutiya *et al.* (7) found that diuron at 10 kg/ha reduced weight, sugar, and vitamin C contents of mandarines (*C. reticulata* Blanco), while Brown and Constantin (2), using bromacil at 3.6 kg/ha in citrus orchards, observed increased yield, soluble solid content, and intensified fruit color in satsumas (*C. unshiu* Marc.) and oranges.

Published information on the action of bromacil and diuron on mineral uptake by citrus species is scarce and contradictory. The objectives of this experiment were (a) to study the effects of two herbicides (bromacil and diuron) and two iron sources ( $\text{FeSO}_4$  and FeEDDHA) on the physiological and morphological status and mineral composition of young sour lime and sour orange seedlings and (b) to

observe any possible interactions existing between these herbicides and nutrients specially with regard to Fe uptake by the plants.

#### MATERIALS AND METHODS

One-year-old seedlings of sour lime [*Citrus aurantifolia* (Christ.) Swing.] and sour orange (*C. aurantium*), propagated from local seeds, were transplanted into 10-liter plastic pots containing 10 kg of a virgin Calcixerollic Xerochrept soil (pH = 8.2, CaCO<sub>3</sub> equivalent = 47.0%, organic matter = 0.31%, total N = 0.02% and extractable Fe = 4.2 ppm) in the greenhouse. The approximate night and day temperatures were 20 and 28°C, respectively, for the entire duration of the experiment. After establishment (5 weeks), the treatments were applied in 250 ml of water to the soil surface of each pot, carefully avoiding direct contact with the plants.

The design of the experiment was a split plot with four replications and with herbicides as the main and iron as the subtreatments. Each plot consisted of one pot containing 2 seedlings. Treatments consisted of 0, 4, and 8 kg/ha bromacil (5-bromo-3-sec-butyl-6-methyluracil); 0, 3, and 6 kg/ha diuron [3-(3, 4-dichlorophenyl)-1, 1-dimethylurea]; and 0, 5, and 10 ppm Fe from either FeSO<sub>4</sub> or FeEDDHA. Herbicides were applied in the commercial range recommended for once-a-year application. After application of the treatments, the plants were grown for 16 weeks.

At the end of the experiment, the seedlings were harvested by cutting the shoots at the soil surface and growth was measured as plant height, leaf number, and leaf area. The leaves and roots were washed in three changes of tap water, and once in distilled water, and were dried at 70°C for 48 hours. For elemental analyses, the dried plant parts were ground and acid digested. Contents of Fe, Mn, Zn, and Mg were determined by an atomic absorption

spectrophotometer and those of Na and K by a flame photometer. Phosphorus, total N, and Cl were determined by ascorbic acid method, micro-Kjeldahl, and dry ashing-silver nitrate titration methods, respectively (5).

Two chlorophyll measurements (a, b, and total) were made at 25 days post-treatment and at harvest on two-leaf disks (1.2 cm in diameter), homogenized in 15 ml of 80% (v/v) acetone, and centrifuged at 8000 rpm for 10 min (1). The absorbances were measured with a spectrophotometer at 490 and 690 m $\mu$ .

#### RESULTS AND DISCUSSION

Plant injury occurred at the lower rates of the herbicides, while death resulted at the higher rates. Herbicides and iron caused significant changes in morphological and physiological characters and also mineral composition of sour lime and sour orange seedlings (Tables 1 and 2 and Figs. 1 and 2).

The different combinations of organic and inorganic Fe with bromacil and diuron resulted in responses varying from growth retardation to synergistic lethal action. Although the general growth responses to bromacil-Fe and diuron-Fe combinations were more or less similar, sour lime was more sensitive and the bromacil-Fe combination had greater inhibitory effects on both species (Table 1).

The lower rate of FeEDDHA and both rates of FeSO<sub>4</sub>, when used in combination with the herbicides, were stimulatory to the chlorophyll and nutrient contents of the leaves of both sour lime and sour orange seedlings as compared with the plants treated with the herbicides alone. Bromacil-Fe or diuron-Fe greatly reduced the amount of chlorophylls a and b in the leaves of both species 25 days post-treatment (Table 2). However, at harvest the recovered plants had a higher chlorophyll content than controls (Fig. 1).

Significant herbicide-Fe interactions were also observed

Table 1. Effect of Fe and herbicide applications on growth of sour lime and sour orange seedlings.

Herbicide	Fe <sup>†</sup>	Sour lime			Sour orange		
		Stem height (cm)	Root length (cm)	Leaf area (cm <sup>2</sup> )	Stem height (cm)	Root length (cm)	Leaf area (cm <sup>2</sup> )
No herbicide	+F1	84 <sup>ab</sup>	61.0 <sup>bc</sup>	923.3 <sup>ab</sup>	98.0 <sup>b-f</sup>	55.5 <sup>bc</sup>	1035.0 <sup>de</sup>
	+F2	98 <sup>abc</sup>	60.0 <sup>bc</sup>	932.5 <sup>ab</sup>	101.2 <sup>c-f</sup>	64.6 <sup>c</sup>	1032.9 <sup>de</sup>
	+F3	105 <sup>bc</sup>	64.7 <sup>bc</sup>	1058.8 <sup>bc</sup>	110.7 <sup>d-f</sup>	67.6 <sup>c</sup>	1081.5 <sup>ef</sup>
	+F4	112 <sup>c</sup>	63.4 <sup>bc</sup>	1056.5 <sup>bc</sup>	117.6 <sup>ef</sup>	66.7 <sup>c</sup>	1221.0 <sup>fg</sup>
	+F5	115 <sup>c</sup>	69.0 <sup>c</sup>	1165.8 <sup>c</sup>	123.4 <sup>f</sup>	68.5 <sup>c</sup>	1362.0 <sup>g</sup>
3 kg/ha diuron	+F1	79 <sup>ab</sup>	48.2 <sup>ab</sup>	1018.4 <sup>abc</sup>	92.1 <sup>a-e</sup>	37.0 <sup>a</sup>	834.2 <sup>bc</sup>
	+F2	76 <sup>a</sup>	52.0 <sup>abc</sup>	931.6 <sup>ab</sup>	89.5 <sup>a-d</sup>	42.5 <sup>ab</sup>	830.0 <sup>bc</sup>
	+F3	77 <sup>a</sup>	45.5 <sup>ab</sup>	940.8 <sup>ab</sup>	93.8 <sup>a-e</sup>	41.0 <sup>ab</sup>	916.0 <sup>cd</sup>
	+F4	74 <sup>a</sup>	40.7 <sup>a</sup>	894.7 <sup>a</sup>	95.4 <sup>a-e</sup>	40.0 <sup>ab</sup>	859.1 <sup>bc</sup>
	+F5	-	-	-	88.4 <sup>a-d</sup>	36.5 <sup>a</sup>	794.9 <sup>abc</sup>
4 kg/ha bromacil	+F1	- <sup>§</sup>	-	-	85.2 <sup>abc</sup>	34.0 <sup>a</sup>	710.0 <sup>ab</sup>
	+F2	-	-	-	78.0 <sup>ab</sup>	36.5 <sup>a</sup>	669.7 <sup>a</sup>
	+F3	-	-	-	78.8 <sup>ab</sup>	32.0 <sup>a</sup>	647.9 <sup>a</sup>
	+F4	-	-	-	75.1 <sup>a</sup>	30.4 <sup>a</sup>	638.6 <sup>a</sup>
	+F5	-	-	-	-	-	-

<sup>†</sup>F1 = no Fe; F2 and F3 = 5 and 10 ppm Fe as FeSO<sub>4</sub>; F4 and F5 = 5 and 10 ppm Fe as FeEDDHA, respectively.

\* Mean separation within columns by Duncan's multiple range test at the 5% level of probability.

<sup>§</sup> Shows death at measurement.

Table 2. Effects of Fe and herbicide applications on chlorophyll content of the leaves ( $\text{mg}/\text{dm}^2$ ) of sour lime and sour orange seedlings 25 days after treatment.

Herbicide	Fe <sup>†</sup>	Sour lime		Sour orange	
		Chlorophyll a	Chlorophyll b	Chlorophyll a	Chlorophyll b
No herbicide	+F1	2.84a-d*	1.28ab	3.67d-h	1.56def
	+F2	3.09b-e	1.57cd	3.79e-h	1.75e-h
	+F3	3.79fg	1.71de	3.98gh	1.76e-h
	+F4	3.85fg	1.57cd	3.91fgh	1.71h
	+F5	4.27g	1.90e	4.14h	1.84h
3 kg/ha diuron	+F1	2.49abc	1.21ab	3.69d-h	1.61d-h
	+F2	3.07b-e	1.27ab	3.42b-f	1.66d-h
	+F3	3.18de	1.34abc	3.56c-g	1.67d-h
	+F4	2.78a-d	1.35abc	3.64c-h	1.73e-h
	+F5	2.45a	1.16a	3.58c-g	1.72e-h
6 kg/ha diuron	+F1	2.48ab	1.27ab	3.30b-e	1.51cde
	+F2	2.98a-e	1.24ab	3.36b-e	1.72e-h
	+F3	2.72a-d	1.28ab	3.76e-h	1.81gh
	+F4	3.12cde	1.36abc	3.24bcd	1.61d-h
	+F5	2.74a-d	1.32abc	3.36b-e	1.72e-h
4 kg/ha bromacil	+F1	2.82a-d	1.32abc	2.98b	1.48bcd
	+F2	2.83a-d	1.25ab	3.24bc	1.54def
	+F3	3.15de	1.33abc	3.17bc	1.57def
	+F4	2.57a-d	1.31abc	3.01b	1.44bcd
	+F5	3.47ef	1.47bcd	2.49a	1.19a
8 kg/ha bromacil	+F1	3.01a-e	1.45bc	3.02b	1.53def
	+F2	2.60a-d	1.20ab	3.04b	1.47bcd
	+F3	2.99a-e	1.27ab	2.49a	1.28abc
	+F4	2.79a-d	1.47bcd	2.47a	1.10a
	+F5	3.18de	1.43abc	2.17a	1.27ab

<sup>†</sup>F1 = no Fe; F2 and F3 = 5 and 10 ppm Fe as  $\text{FeSO}_4$ ; F4 and F5 = 5 and 10 ppm Fe as FeEDDHA, respectively.

\* Mean separation within columns by Duncan's multiple range test at the 5% level of probability.

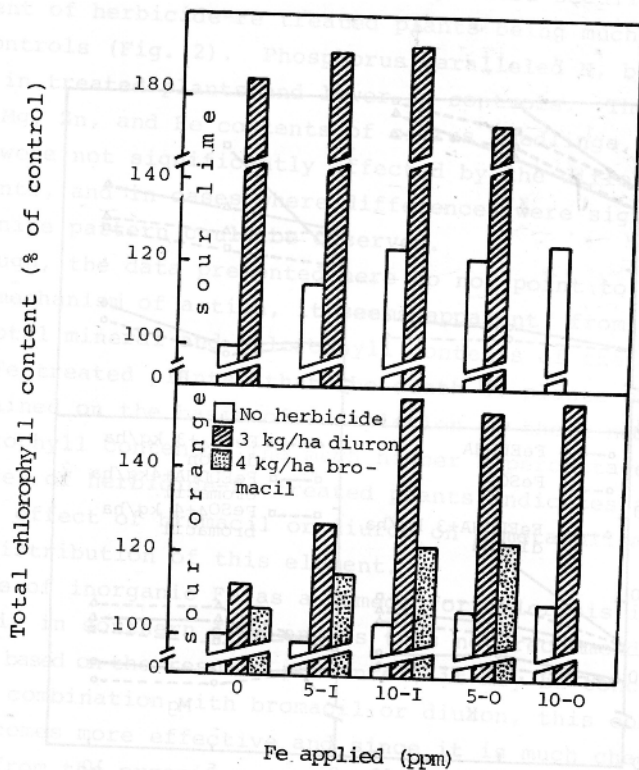


Fig. 1. Effects of Fe and herbicide treatments on total chlorophyll content of sour lime and sour orange leaves at harvest. O = no Fe; 5-I and 10-I = 5 and 10 ppm Fe as  $FeSO_4$ ; 5-O and 10-O = 5 and 10 ppm Fe as FeEDDHA.

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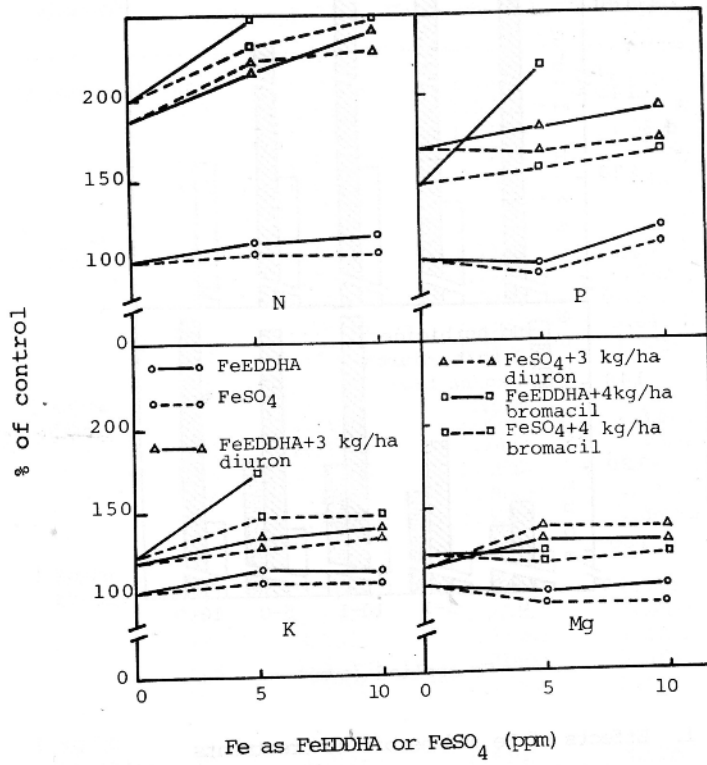


Fig. 2. Effects of Fe and herbicide treatments on N, P, K, and Mg contents of leaves of sour orange seedlings.



for mineral nutrition of the species. The N content of the leaves was found to be related to the Fe concentration used for both the herbicide treated and untreated seedlings; the N content of herbicide-Fe treated plants being much higher than controls (Fig. 2). Phosphorus paralleled N, being higher in treated plants and lower in controls. The Cl, Na, K, Mg, Zn, and Fe contents of citrus seedlings, in most cases, were not significantly affected by the different treatments, and in cases where differences were significant, no definite pattern could be observed.

Although, the data presented here do not point to a definite mechanism of action, it seems apparant, from the large total mineral and chlorophyll contents of the herbicide-Fe treated plants, that the death of plants can not be explained on the basis of a depletion of these nutrients or chlorophyll content. The much higher N percentage in the leaves of herbicide-Fe treated plants indicates a possible effect of bromacil or diuron on N metabolism or on the distribution of this element.

The use of inorganic Fe as a remedy for chlorosis is ineffectiv in southern Iran and is thus not recommended. However, based on the results presented, it may be concluded that, in combination with bromacil or diuron, this compound becomes more effective and since it is much cheaper than Fe from the organic source, its use may be recommended. Since both bromacil and diuron are currently registered for use in citrus orchards, this practice has the additional advantage of increasing the activity of the herbicide on weeds, while the older trees being more resistant than seedlings, escape injury.

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