THE EFFECT OF THE DOMINANCE OF A METABOLIC SYSTEM REQUIRING IRON OR COPPER ON THE DEVELOPMENT OF LIME-INDUCED CHLOROSIS

J. C. BROWN

Bureau of Plant Industry, Beltsville, Maryland

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In an earlier experiment Brown and Hendricks (**1**) grew both chlorosis-susceptible plants and plants not susceptible to chlorosis on a calcareous soil of limited iron-supplying capacity, an organic soil of limited copper-supplying capacity and mixtures of the two soils. Since it was believed that a deficiency of either element in the plant would result in a changed activity of some enzyme, comparative enzymatic activities of ascorbic acid oxidase (a copper enzyme), catalase, and peroxidase (iron enzymes) were determined. Three varieties of lupines and one of soybeans developed a severe lime-induced chlorosis but showed no response to copper. These plants all exhibited comparatively low catalase activity. Lime-induced chlorosis did not develop in Thatcher wheat grown on the naturally calcareous soil, but the crop did show copper-deficiency symptoms when grown on the organic soil. Ascorbic acid oxidase activity was high in normal Thatcher wheat, and since this is believed to be the terminal oxidase in wheat (**7**), it may be a factor which allows the plant to grow on a soil with limited iron supplying capacity.

In the present experiment it was considered desirable to extend the above observations to additional crops to determine the extent to which this generalization might hold. A classification of plants seemed possible based upon the plant's response to the two types of nutrition studied.

Methods and materials

Thatcher spring wheat, Hannchen barley, Long Green okra, cocklebur, Ottawa Select spring rye, Red kidney beans, Marglobe tomatoes, and Connecticut broadleaf tobacco were grown on the following soils: soil 1, naturally calcareous, pH 7.8; soil 2, two thirds calcareous and one third organic, pH 7.5; soil 3, one third calcareous and two thirds organic, pH 7.2; soil 4, organic soil, pH 6.0; and soil 5, organic with added $CuSO_4 \cdot 5\,H_2O$. These soils provided a range of availability of both iron and copper. The plants were grown in the greenhouse on the above soil treatments in two-gallon glazed crocks as previously described (**1**).

Comparative measurements of the apparent activity of the enzymes ascorbic acid oxidase, catalase, and peroxidase were carried out under fixed procedures previously described (**1**). Ascorbic acid oxidase activity was determined manometrically and catalase gasometrically, using ascorbic acid and hydrogen peroxide, respectively, as substrates. Peroxidase activity was determined colorimetrically using catechol. All plant material was harvested for analyses about 8:00 A.M. The leaves were taken from the upper half of the plant, combined, and cut into small pieces with scissors. Samples for ascorbic acid oxidase and peroxidase measurements were frozen overnight and determined the day following harvest. Catalase measurements were completed approximately two hours after harvest.

Comparative rather than quantitative extraction of the enzymes from the plant was considered satisfactory to the objectives of this investigation. Several duplicated experiments showed that the error between extractions of enzyme from the plant tissue was about 15%. The agreement of the extractions appeared to warrant comparisons between like material grown on the varied soil treatments. The plants were all grown, harvested, and the determinations made under like conditions.

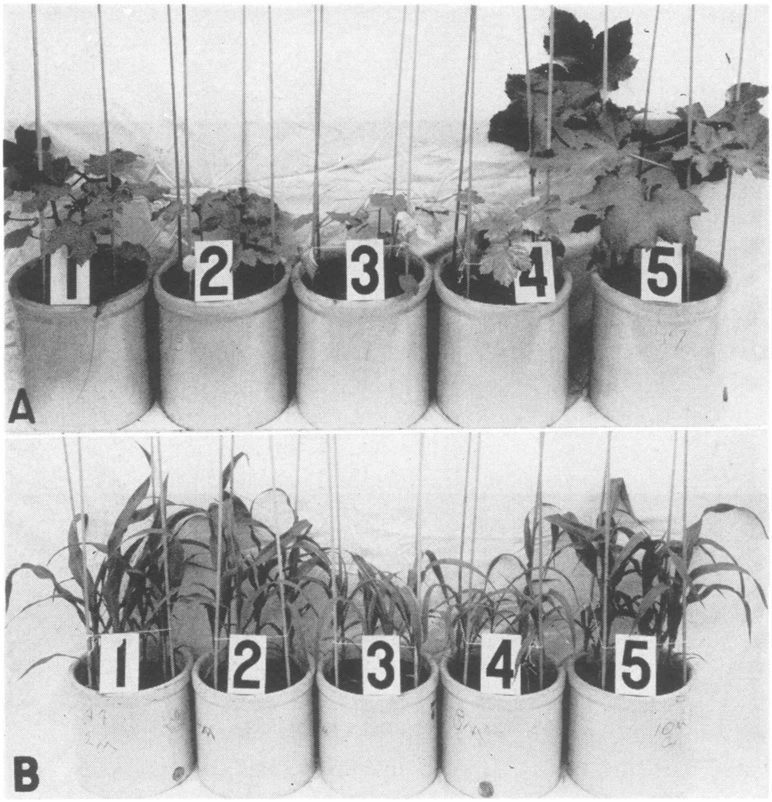

FIG. 1. A. Long Green okra on soils 1 to 5. Plants with limited copper supply on soils 3 and 4 and to a lesser extent soil 2 show interveinal chlorosis of lower leaves and retarded growth. B. Midland milo on soils 1 to 5. Plants with limited copper supply on soils 3 and 4 show a reddening of leaf tips, chlorosis and retarded growth.

Results

Wheat, barley, okra, milo, and cocklebur plants responded to copper but did not develop lime-induced chlorosis. Figures 1 A and B, and 2 A show some of these plants and the nature of their response. Red kidney beans (fig. 2 B) and tobacco grew vegetatively well on the copper-deficient soil but did show some lime-induced chlorosis when grown on the calcareous soil. No chlorosis was evident in the spring harvested tobacco, but chlorosis did develop in the fall grown crop. None of the five soil treatments caused rye (fig. 3) to become chlorotic although the yield of plants grown on the calcareous soil was reduced.

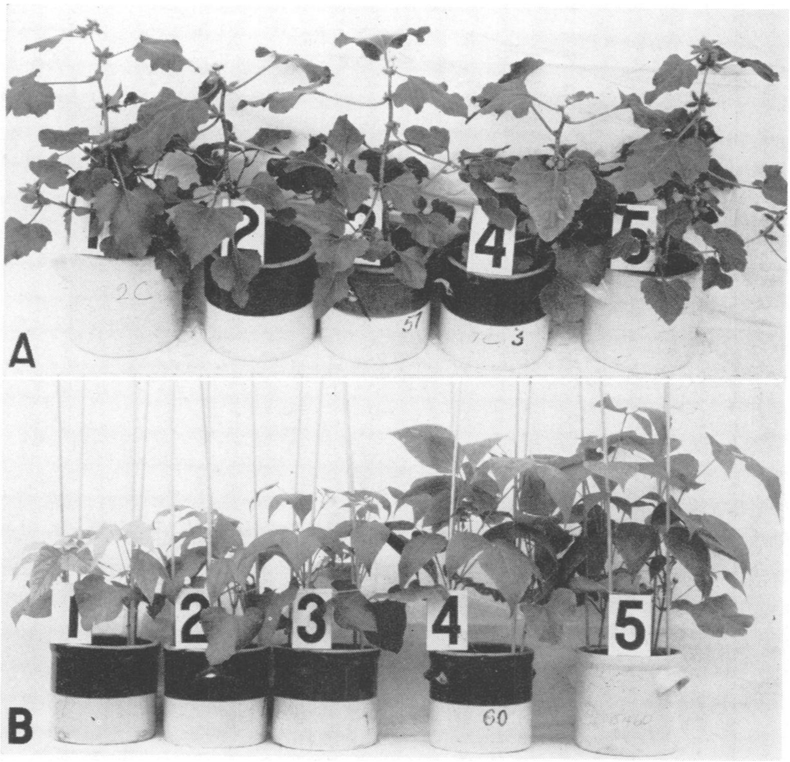

FIG. 2. A. Cocklebur grown on soils 1 to 5. Plants with limited copper supply on soils 3 and 4 failed to develop their burs and the leaves show necrotic areas. B. Red kidney beans grown on soils 1 to 5. Plants grown on calcareous soil 1 are chlorotic and retarded in growth. Plants grown with limited copper supply on soils 3 and 4 did not respond to copper.

Tables I and II give the yields and comparative enzymatic data for the above plants. The ascorbic acid oxidase activity was generally in agreement with the copper level of the growth medium regardless of the plant's showing a growth response to copper. Those plants which developed lime-induced chlorosis generally had a lower catalase activity than the nonchlorotic plants grown on the other treatments. Yield and enzymatic activity of tobacco appeared to be influenced by the season in which grown (fall or spring).

The visual deficiencies and enzymatic activities were not the same for all plants which responded to copper. Vegetatively, cocklebur showed very

TABLE I

YIELD AND RELATIVE ENZYME ACTIVITY OF WHEAT, BARLEY, MILO, OKRA, AND COCKLEBUR GROWN ON SOILS OF DIFFERENT IRON- AND COPPER-SUPPLYING CAPACITIES. ALL OF THESE PLANTS RESPONDED TO COPPER.

Crop (spring 1952)	Age	Soils									
		1 Calcareous		2 Two thirds calcareous one third organic		3 One third calcareous two thirds organic		4 Organic		5 Organic plus Copper	
	days										
						Catalase					
Thatcher sp. wheat	42	427		393		310*		241*		458	
Hannchen barley	44	181		228		199		193		272	
Hannchen barley	51	189		317		223*		169*		212	
Midland milo	....	61		68		70*		104*		112	
Long Green okra	48	203		202*		198*		192*		186	
Cocklebur	55	100		190		180*		180*		130	
						Peroxidase					
Milo	....	32		33		23*		36*		46	
Okra	48	74		68*		81*		57*		79	
Cocklebur	55	58		47		58*		49*		71	
						Ascorbic acid oxidase					
		Leaves	Nodes	Leaves	Nodes	Leaves	Nodes	Leaves	Nodes	Leaves	Nodes
Spring wheat	42	23	163	15	49	7*	32*	5*	15*	24	118
Barley	44	9	74	8	31	−2	33	−4	29	9	66
Barley	51	....	99	....	87	....	50*	....	1*	....	92
		Leaves		Leaves		Leaves		Leaves		Leaves	
Milo	....	15		34**		46**		29**		98	
Okra	48	97		69*		18*		15*		228	
Cocklebur	55	108		108		59*		45*		117	
						Average yield/plant† (Green wt.)					
Spring wheat	42	23		15		12*		20*		33	
Barley	44	29		25		26		24		43	
	51	37		32		24*		21*		47	
Milo	....	22		19		13*		13*		41	
Okra	48	31		8*		5*		8*		96	
Cocklebur											
Stem	55	26		39		44		43		37	
Leaves	55	39		47		55		62		49	
Burs	55	36		21		6*		5*		60	
Total weight		101		107		105		110		146	

*Severe copper deficiency symptoms.

**Endogenous activity very high.

†Average yield/10 plants for wheat and barley.

TABLE II

YIELD AND RELATIVE ENZYME ACTIVITY OF RYE, RED KIDNEY BEANS, TOMATOES, AND TOBACCO GROWN ON SOILS OF DIFFERENT IRON- AND COPPER-SUPPLYING CAPACITIES.

Crop	Date	Age	Soils 1 Calcareous	2 Two thirds calcareous one third organic	3 One third calcareous two thirds organic	4 Organic	5 Organic plus Copper
		days					
					Catalase		
Rye	March 26, 1952	46	350	365	343	261	300
Red kidney beans	March 12, 1952	33	153*	422	451	560	401
Tomatoes	May 6, 1952	62	70*	121	150	128	76
Tobacco	December 31, 1951	59**	188*	412	485	550	267
Tobacco	May 6, 1952	56**	192	208	212	190	103
					Peroxidase		
Rye	March 26, 1952	46	81	64	43	39	76
Red kidney beans	March 12, 1952	33	....	....	....	....	....
Tomatoes	May 6, 1952	62	14*	22	17	16	20
Tobacco	December 31, 1952	59**	33*	45	58	50	59
Tobacco	May 6, 1952	56**	38	56	43	43	37
					Ascorbic acid oxidase		
			Leaves Nodes	Leaves Nodes	Leaves Nodes	Leaves Nodes	Leaves Nodes
Rye	March 26, 1952	46	16 81	22 64	22 43	19 39	20 76
			Leaves	Leaves	Leaves	Leaves	Leaves
Red kidney beans	March 12, 1952	33	232*	160	91	9	336
Tomatoes	May 6, 1952	62	178*	125	98	57	123
Tobacco	December 31, 1951	59**	321*	313	79	6	303
Tobacco	May 6, 1952	56**	143	62	24	27	213
					Average yield/plant† (Green wt.)		
Rye	March 26, 1952	46	18	19	21	31	31
Red kidney beans	March 12, 1952	33	25*	30	34	69	69
Tomatoes	May 6, 1952	62	74*	105	120	156	230
Tobacco.	December 31, 1951	59**	350*	547	607	521	528
Tobacco	May 6, 1952	56**	746	754	855	851	885

*Lime-induced chlorosis.

**Days after transplanting.

†Average yield/10 plants for rye.

FIG. 3. Ottawa Select spring rye grown on soils 1 to 5. Rye did not develop deficiency symptoms on any of the treatments.

little response to copper, but as the plant became reproductive the burs failed to develop where copper was limited. Cocklebur thus differed from wheat, barley, okra, and milo which were all markedly reduced in growth. A very high endogenous activity was observed in determining ascorbic acid oxidase activity in copper-deficient milo plants. A poor correlation (table I) between visual deficiency symptoms and ascorbic acid oxidase activity was obtained for milo grown on soil treatments 1, 2, and 3. Treatments 4 and 5

TABLE III

PLANTS GROWN ON A CALCAREOUS AND ORGANIC SOIL, AND THE OCCURRENCE OF LIME-INDUCED CHLOROSIS OR COPPER-DEFICIENCY SYMPTOMS, RESPECTIVELY.

Plants	Calcareous soil (Lime-induced chlorosis)	Organic soil (Copper-deficiency symptoms)
Thatcher wheat	0	+
Hannchen barley	0	+
Long Green okra	0	+
Midland milo	0	+
Cocklebur	0	+
Red kidney beans	+	0
White lupine	+	0
Blue lupine	+	0
Yellow lupine	+	0
PI-54619-5-1 soybean	+	0
Connecticut broadleaf tobacco	+	0
Marglobe tomatoes	+	++
Minn Hybrid no. 800 corn	+	++
Hawkeye soybean	0	0
Ottowa Select spring rye	0	0

+ Visual deficiency symptoms other than retarded growth.
0 No visual deficiency symptoms.
++ Partial deficiency symptoms.

gave a good correlation. Milo appears to be limited in growth through a copper system, but not necessarily in the same manner as wheat and barley.

Discussion

The plants grown in this and a previous study (**1**) are grouped, table III, according to their response to the two types of nutrition investigated. From this grouping of plants it would appear that the plant's capacity to adjust to a particular environment might largely depend upon the plant's metabolic requirements. Wheat, barley, okra, milo, and cocklebur plants developed severe copper-deficiency symptoms, but none of these plants became chlorotic when grown on the calcareous soil. In contrast to the above plants, three lupines, soybeans, red kidney beans, tobacco, and rye did not respond to copper. This does not preclude some response to copper which might have been observed if these plants had been allowed to complete their reproductive development. The latter plants, except rye and soybeans, developed lime-induced chlorosis and appeared to be much more dependent upon available iron than copper.

Comparative enzymatic activities of ascorbic acid oxidase, catalase, and peroxidase, determined in this investigation, agreed with data from the previous study (**1**). The catalase activity was lower in lime-induced chlorotic than in normal green plants and there was no significant change in the peroxidase activity. If catalase activity is accepted as a possible index of active iron available in plant metabolism (**2**) lime-induced chlorosis appeared to be a true iron deficiency. Also, in view of the data available (**3, 5, 6**) which indicate that the iron concentration in chlorotic plants is about the same as that found in non-chlorotic plants, it seems probable that iron must be inactivated in some lime-induced chlorotic plants.

The cause of this inactivation or nonutilization of the iron present in the plant is yet to be determined. It seems possible that a different or altered metabolism in the plant may be a factor in determining the degree of susceptibility of plants to lime-induced chlorosis, since copper-requiring systems of metabolism seemed to predominate in most plants not susceptible to the chlorosis. The terminal oxidase in wheat and barley, two plants not susceptible to lime-induced chlorosis, has been determined by WAYGOOD (**7**) and JAMES and CRAGG (**4**), respectively, to be ascorbic acid oxidase which might be a factor to enable these plants to utilize iron and grow on the naturally calcareous soil on which active iron becomes limiting in the chlorosis-susceptible plants. Iron-requiring metabolic systems appeared to predominate in plants susceptible to the lime-induced chlorosis and these plants were not copper responsive.

Plants grown on the calcareous soil seemed to have sufficient available copper as indicated by the relatively high ascorbic acid oxidase activity found in most of these plants. This available copper supply, in contrast to a low catalase activity or nonutilizable iron supply, may be an accentuating factor toward the development of lime-induced chlorosis. The average copper concentration in the leaves of 10 of the plants listed in table III was

6 p.p.m. in plants grown on the calcareous soil, 3 p.p.m. in plants grown on the organic soil, and 11 p.p.m. in plants grown on the organic soil to which copper sulphate was added (unpublished). Ascorbic acid oxidase activity appeared to be a good index to the available copper in most plants whether the plants did or did not show visual copper-deficiency symptoms. An increased activity of ascorbic acid oxidase with the addition of copper sulphate to the organic soil was observed in all of the plants studied.

Summary

Wheat, barley, Midland milo, okra, and cocklebur plants responded to copper when grown on the organic soil, but did not develop chlorosis on the calcareous soil. Red kidney beans and tobacco grew well on the copper-deficient organic soil, but did develop chlorosis on the calcareous soil. Tomatoes grown on the calcareous soil became chlorotic and also showed a small response to copper. Rye grew well on all of the soil treatments.

The catalase activity was lower in lime-induced chlorotic plants than in normal green plants but there was no significant change in the peroxidase activity. Ascorbic acid oxidase in the chlorotic plants was about the same as that in the normal plants.

Ascorbic acid oxidase activity appeared to be a good index of the available copper supply, whether the plant did or did not show visual copper-deficiency symptoms.

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LITERATURE CITED

1. Brown, J. C. and Hendricks, S. B. Enzymatic activities as indications of copper and iron deficiencies in plants. Plant Physiol. **27**: 651–660. 1952.
2. Brown, J. C. and Steinberg, R. A. Iron and copper enzymes in leaf lamina of tobacco when deficient in micronutrients or grown on calcareous and organic soils. Plant Physiol. **28**: 488–494. 1953.
3. Iljin, W. S. Metabolism of plants affected with lime-induced chlorosis (calciose). III. Mineral elements. Plant and Soil **4**: 11–28. 1952.
4. James, W. O. and Cragg, J. M. The ascorbic acid system as an agent in barley respiration. New Phytol. **42**: 28–44. 1943.
5. McGeorge, W. T. Nutrient interrelations in lime-induced chlorosis as revealed by seedling tests and field experiments. Arizona Tech. Bull. 116. 1948.
6. Twyman, E. S. The iron and manganese requirements of plants. New Phytol. **50**: 210–226. 1951.
7. Waygood, W. E. Physiological and biochemical studies in plant metabolism. II. Respiratory enzymes in wheat. Canadian Jour. Res. C **28**: 7–62. 1950.