MANGANESE TOXICITY of LEGUMES Seeded in Kentucky Strip-Mine Spoils

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SINCE SPOILS usually lack nitrogen, legumes appear to be a logical choice for quick cover because they are able to convert nitrogen from the air to nitrogen needed for plant growth. However, legumes do not grow well on acid spoils. Many studies of legumes seeded on soils indicate that poor growth under acid conditions often can be attributed to toxicities caused by excess manganese (Mn) and aluminum brought into solution by the acid conditions *(Jackson 1967)*.

The objectives of our study were to investigate whether Mn toxicity occurred on legumes grown on Kentucky strip-mine spoils, whether the amount of toxicity varied among spoils, and whether the toxic effects differed among legume species; and then to find chemical characteristics of the spoil that we can use to predict the occurrence of the toxicity.

Before we could achieve these objectives, we had to know what the Mn toxicity symptoms on the legumes are. Chlorosis on leaf margins was the outstanding Mn toxicity symptom on Korean lespedeza described by Morris *(1948)* and Morris and Pierre *(1949)*. We could not find descriptions of Mn toxicity on the other species used in this study, although a marginal chlorosis was described as a Mn toxicity symptom on red clover by Hewitt *(1946)* and on alfalfa by Ouellette and Dessureaux *(1958)*. Therefore, we produced known Mn toxicity symptoms by growing the legumes in nutrient solutions and then looked for these symptoms on the same species grown in spoils.

METHODS

Three of the species studied, Korean lespedeza (*Lespedeza stipulacea* Maxim.), Kobe lespedeza (*L. striata* (Thunb.) H. & A.), and sericea lespedeza (*L. cuneata* (Dumont) G. Don), are the legumes usually seeded for ground cover on spoils in eastern Kentucky and adjacent areas. We also studied bicolor lespedeza (*L. bicolor* Turcz. 'Natob'), a shrub that has possibilities for wildlife food and cover on spoils; birdsfoot trefoil (*Lotus corniculatus* L. 'Viking'), a species that has grown well on acid spoils in other states *(Struthers 1960)* and in our field studies; and black locust (*Robinia pseudoacacia* L.), which is the tree most commonly planted on spoils in this area.

Nutrient Solution Cultures

Mn toxicity symptoms were produced by germinating and growing the legumes in rafts floating on nutrient solutions. Rafts were made from styrofoam 1 cm. thick, into which 1.5-cm. diameter holes were punched; glass wool was then inserted into the holes. Seeds were placed on the glass wool and the rafts were floated on nutrient solution in 700-ml. polyethylene containers. The nutrient solution was the Hoagland Number 1 *(Hoagland and Arnon 1950)*; the iron was modified to 0.2 ppm. supplied as sodium ferric diethylenetriamine pentaacetate (Fe DTPA). Treatments were 0.5 ppm. Mn, which is the normal Mn concentration for Hoagland's solution, and 20 ppm. Mn. Solutions were not aerated but were replaced daily. The pH of the nutrient solution was 4.7. This study was conducted in the greenhouse in March and April. There were two replications of each treatment. In a later study the legumes were grown in nutrient solutions containing 0.25, 0.5, 1, 2, 4, 8, and 12 ppm. Mn.

The technique worked well for producing symptoms caused by excess Mn. However, we could not take quantitative data because we encountered difficulties with algae growing in the nutrient solution.

The excess Mn treatments produced a chlorosis on leaf margins of all the species. The contrast between the chlorotic and non-chlorotic areas was pronounced on all species except Kobe lespedeza. For example, the Munsell color notation was 2.5 GY 7/6 on the chlorotic margin and 7.5 GY 5/6 on the unaffected portion of a Korean lespedeza leaflet. On Kobe lespedeza the marginal chlorosis gradually blended into the greener color of the leaflet center.

Small dusky-red spots (10 R 2/2)—a secondary toxicity symptom — appeared in the chlorotic areas on Korean and Kobe; with time the spots turned brown (necrotic). Necrotic spots also developed in the chlorotic areas on the sericea and bicolor lespedeza.

Pot Tests

Samples of 46 spoils from 11 different Kentucky coal strip-mining operations were collected in the fall of 1966. Spoils associated with these coal seams were sampled: Hindman (Hazard 9), Lily, Fireclay (Dean), Harlan, Francis, Mason, Number 11, and Number 12.

Spoils were selected to give a range in pH from neutral to extremely acid. Problems with unoxidized sulfides were minimized by only collecting spoils that had been exposed 6 months or longer. Surface accumulation of salts was avoided by excluding the surface 3 inches of spoil. Spoils were screened and fragments larger than 1.3 cm. diameter were discarded. After it was dried, each spoil was thoroughly mixed, and 1,800 grams was weighed into pots. Since phosphorus is deficient on many spoils, all spoils were fertilized at the rate of 100 ppm. P by adding monobasic calcium phosphate in suspension to each pot and then mixing. Six pots of each spoil were thus treated, and individual pots were seeded to one of the six legumes. The appropriate commercial inoculant was used on all seeds with the exception of locust, which was treated with a small amount of soil from beneath a locust stand. The species treatments were not replicated.

The pots were seeded and placed in the greenhouse on October 3, 1966. The weather in October was generally sunny; there were some cloudy days in November. Artificial lighting was used to give a 12-hour day. Pots were weighed twice a week and distilled water was added to bring them to approximate field capacity; at other times water was added as needed. Notes were taken on the extent of marginal chlorosis on the first leaves that emerged and at weekly intervals thereafter. The degree of chlorosis was rated: slight — up to 10 percent of leaf chlorotic; moderate — 10 to 40 percent of leaf chlorotic; and severe — over 40 percent of leaf chlorotic.

The Korean, Kobe, and black locust grew fast on some spoils and were harvested on all spoils after 40 days growth. The sericea, birdsfoot trefoil, and bicolor grew slower and were harvested after 60 days. When harvested, plants were cut off just above the cotyledons, oven-dried at 70°C. for 24 hours, and then weighed.

After the tops were harvested, the roots were examined, and nodulation was rated as *none, some,* or *good*. Included in the *none* rating were pots where less than half of the plants had one or two very small nodules and the remaining plants had none. Rating nodulation as *good* meant that numerous nodules (varying somewhat among species) occurred on the primary root, and some nodules occurred throughout the rest of the root system. Nodules in the *good* rating were mostly medium to large as compared to the smaller nodules in the *none* rating. (Nodulation of legumes grown in spoils with pH above 5.5 was used as the standard for judging *good* nodulation.) The intermediate condition of nodulation was rated as *some*.

Spoil pH was determined with a pH meter on a 2 to 1 distilled water-to-spoil mixture that was stirred, allowed to settle 30 minutes, and stirred again before the electrodes were immersed into the supernatant liquid. The pH was determined on spoil samples when the study was begun and later on spoils in which the legumes had grown. No spoil pH varied more than 0.3 pH unit from the initial reading and many were the same before as after cropping. The initial pH values (appendix, table 4) were used when the plant growth data were analyzed.

Water-soluble Mn and calcium were extracted from 20-g. samples of spoils shaken for 30 minutes in 100 ml. of distilled water. After filtration, Mn and calcium were determined by atomic absorption spectroscopy. When determining calcium, all samples and standards were made up to include 1,500 ppm. strontium to prevent interferences. Exchangeable Mn and aluminum were extracted from 20-g. spoil samples by 30 minutes shaking in 100 ml. of 1 N KCl. After filtering, Mn was determined on the extract by atomic absorption spectroscopy; aluminum was determined by the cyanine R method.

RESULTS & DISCUSSION

Chlorosis on leaf margins of legumes grown on the spoils varied from none to severe. The legume species also varied in susceptibility. Korean, sericea, and bicolor lespedeza seedlings developed chlorosis on many more spoils than did Kobe lespedeza, birdsfoot trefoil, or black locust.

When marginal chlorosis occurred it was present on the first leaf or leaflets that emerged after the cotyledons opened. With very severe chlorosis (70 to 100 percent of leaf surface) no further

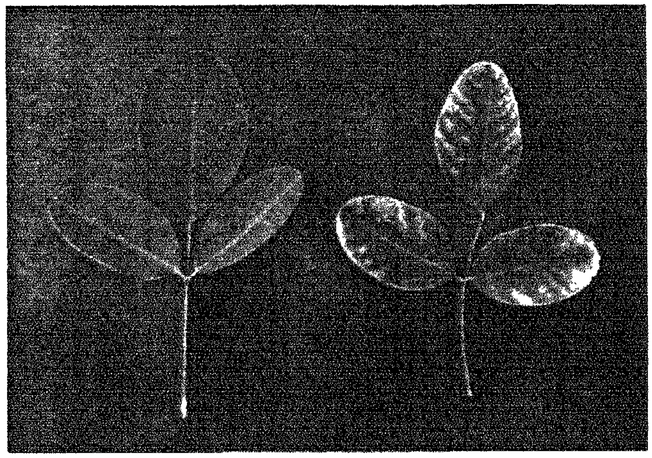

Figure 1. — Bicolor lespedeza leaf on the right shows marginal chlorosis indicating manganese toxicity. Leaf on the left is from an unaffected plant.

Figure 2. — Manganese toxicity symptoms on sericea lespedeza leaflets as shown by chlorosis on leaf margins. None (left), moderate (center), and severe (right).

growth occurred. The chlorotic margins were distinct (figures 1 and 2) on all species except Kobe lespedeza and birdsfoot trefoil. On Kobe the chlorosis gradually became more obvious at the margins. Chlorosis on birdsfoot trefoil was usually on the margins, but it sometimes appeared in irregular spots near the leaf margins.

Small red spots appeared in the chlorotic areas of Korean and Kobe lespedeza leaves and the spots later turned brown. Brown (necrotic) spots also appeared in the chlorotic areas on the sericea, bicolor, and on the more chlorotic birdsfoot trefoil.

These symptoms are the same as those produced in the nutrient solutions where excess Mn was present. The Mn toxicity symptoms on Korean lespedeza as described by Morris and Pierre *(1949)* are also like those symptoms observed in this study. Thus, we assume that Mn toxicity is responsible for the marginal chlorosis and necrotic spotting on the legumes grown on the spoils.

In some spoils, sericea and black locust that initially had light to moderate marginal chlorosis later produced new leaves with no chlorosis. This ability to overcome the toxicity was not noted with the other species.

We have observed the Mn toxicity symptoms on young leaves in field plantings of all the species used in this study.

Yields generally decreased as the degree of chlorosis increased (table 1). A rigorous comparison of the yields with degree of chlorosis cannot be made because aluminum toxicity as well as Mn toxicity undoubtedly inhibits plant growth on many of the acid spoils. Stubby roots with few laterals, a known characteristic of aluminum toxicity, were found on plants grown in some of the spoils; however, incipient aluminum toxicity has no easily recognized symptoms. Differences in plant-available nutrients — particularly nitrogen — among spoils also may have affected yields.

Korean and bicolor lespedeza usually developed symptoms of Mn toxicity when grown on spoils with pH below 5, and occasion-

Table 1. — Relative yields of legumes with given degrees of chlorosis. Observations were included from all spoils from which live plants were harvested

Species	Degree of chlorosis			
	None	Slight	Moderate	Severe
Korean lespedeza	1.0	0.73	0.44	0.15
Kobe lespedeza	1.0	.57	.45	.15
Sericea lespedeza	1.0	.50	.31	.23
Bicolor lespedeza	1.0	.70	.38	.27
Birdsfoot trefoil	1.0	.27	.22	--
Black locust	1.0	.58	.68	.05

Table 2. — Number of spoils in given pH ranges producing manganese toxicity symptoms on legumes

Species	pH range			
	4.0 to 4.4 (11)*	4.5 to 4.9 (12)*	5.0 to 5.4 (11)*	Over 5.4 (4)*
Korean lespedeza	10	8	3	0
Kobe lespedeza	8	2	0	0
Sericea lespedeza	11	8	0	0
Bicolor lespedeza	11	11	4	0
Birdsfoot trefoil	5	1	0	0
Black locust	5	1	0	0

* Basis: Number of spoils in grouping.

ally on spoils with pH in the 5.0 to 5.4 range (table 2). Sericea lespedeza in the seedling stage was also susceptible to Mn toxicity below this pH range, but on some spoils it outgrew the toxicity symptoms. Kobe lespedeza, birdsfoot trefoil, and black locust seldom developed Mn toxicity symptoms on spoils with a pH above 4.4. Thus it appears that spoil pH can be used as a guide for predicting Mn toxicity on legume species seeded in spoils.

Water-soluble Mn in the spoils was not useful in predicting Mn toxicity on the legumes. For example, Korean lespedeza had toxicity symptoms when grown on various spoils containing 1 to 50 ppm. water-soluble Mn, but showed no symptoms when grown on other spoils with as much as 30 ppm. water-soluble Mn. Black locust and birdsfoot trefoil had no toxicity symptoms when grown on the three spoils containing 50 ppm. water-soluble Mn.

Water-soluble Mn in spoils probably does not predict Mn toxicity because high concentrations of soluble calcium and magnesium in some spoils limit Mn uptake by plants *(Hewitt 1946, Ouellette and Dessureaux 1958)*. However, it is not a simple Mn to calcium relationship because the degree of chlorosis could not be related to the ratio of water-soluble calcium to water-soluble Mn.

Nodulation of the legumes also followed definite patterns in relation to spoil acidity. At pH below 4.5, little nodulation occurred on the seedlings (table 3). The pH range 4.5 to 4.9 was a border zone where chances for good nodulation on black locust

Table 3. — Number of spoils in given pH range growing legumes having good nodulation

Species	pH range		
	4.0 - 4.4 (11)*	4.5 - 4.9 (12)*	5.0 - 5.4 (11)*
Korean lespedeza	0	6	10
Kobe lespedeza	1	7	11
Bicolor lespedeza	0	4	10
Sericea lespedeza	0	5	10
Birdsfoot trefoil	1	6	11
Black locust	0	8	10

* Basis: Number of spoils in grouping.

were favorable, and chances for good nodulation on the remainder of the species were fair. At a pH of 5 and above nodulation was usually good on all species. Keep in mind that phosphorus was added to these spoils. Legume growth and nodulation is limited on many spoils by very low amounts of plant-available phosphorus.

Future work on suitability of plants for strip-mine revegetation should include screening not only among but within species for tolerance to toxicities caused by acid conditions. Differences have been found in tolerance to excess Mn among selections of Korean lespedeza *(Morris and Pierre 1949)* and alfalfa *(Ouellette and Dessureaux 1958)*. Foy and his co-workers *(1965, 1967)* are now working intensively on selection of acid-tolerant varieties within agronomically important species.

From this study we conclude that spoil pH is the best guide for predicting Mn toxicity of legumes seeded in spoil. Our results indicate that Korean, sericea, and bicolor lespedeza should be restricted to spoils where pH is above 5.0. However, sericea outgrew the early toxicity symptoms and developed normally on some of the spoils in the 4.5 to 5.0 pH range. The other more Mn-tolerant legumes — Kobe lespedeza, birdsfoot trefoil, and black locust — may be considered for seeding on spoils with pH down to 4.5, although success in stand establishment may be limited by poor or no nodulation on some spoils with pH below 5.0.

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APPENDIX

Table 4. — Chemical characteristics of spoils used in manganese toxicity study

Spoil number	Associated coal seam	pH	Exchangeable — Aluminum	Exchangeable — Manganese	Water soluble — Manganese	Water soluble — Calcium
			ppm.	*ppm.*	*ppm.*	*ppm.*
1	Fireclay	4.5	36	72	11.5	340
2	Fireclay	4.5	54	68	3.3	61
3	Fireclay	3.5	175	115	25.0	525
4	Fireclay	4.0	150	126	23.7	175
5	Fireclay	3.5	300	299	118.0	450
6	Fireclay	3.4	275	80	22.0	420
7	Lily (1)**	3.8	175	23	1.5	3
8	Lily (1)	6.4	2	2	.2	59
9	Lily (1)	5.1	24	29	.2	2
10	Mason	4.1	75	110	16.3	70
11	Mason	4.1	130	58	4.5	26
12	Mason	4.4	200	51	2.3	5
13	Mason	4.2	65	46	8.3	97
14	Mason	5.2	4	14	2.3	61
15	Mason	4.9	26	15	.5	2
16	Francis	4.3	300	66	6.0	23
17	Francis	4.8	90	30	2.3	7
18	No. 11 & 12 (1)	5.1	1	65	23.0	960
19	Hindman (1)	3.6	118	68	26.0	560
20	Hindman (1)	5.4	12	69	3.5	35
21	Hindman (1)	4.2	53	87	26.0	480
22	Hindman (1)	4.7	180	59	.5	2
23	Lily (2)	5.1	13	67	3.0	35
24	Lily (2)	3.8	140	128	16.0	95
25	Lily (2)	4.2	59	171	26.0	160
26	Lily (2)	4.5	33	50	8.0	290
27	Hindman (2)	5.4	60	45	.2	2
28	Hindman (2)	3.9	275	90	8.5	62
29	Hindman (2)	5.3	90	23	.2	2
30	Hindman (2)	4.8	180	46	.2	2
31	Hindman (2)	5.0	26	9	.2	6
32	Hindman (3)	3.8	103	117	52.0	3,000
33	Hindman (3)	6.2	2	17	6.8	2,240
34	Hindman (3)	4.2	71	94	18.1	850
35	Lily (3)	5.4	20	55	.5	8

** Stripping operation number.

CONTINUED

Table 4. — *continued*

Spoil number	Associated coal seam	pH	Exchangeable —		Water soluble —	
			Aluminum	Manganese	Manganese	Calcium
			ppm.	*ppm.*	*ppm.*	*ppm.*
36	Lily (3)	4.9	150	72	.5	3
37	Lily (3)	4.8	22	142	9.8	51
38	Lily (3)	5.3	5	126	2.3	25
39	Hindman (4)	3.0	250	240	175.0	1,840
40	Hindman (4)	7.1	2	2	.5	670
41	Hindman (4)	4.5	75	60	1.8	56
42	Harlan	5.5	27	17	0.0	7
43	Harlan	4.2	200	30	3.5	34
44	Harlan	4.6	42	21	1.0	25
45	No. 11 & 12 (2)	4.9	18	97	53.0	2,800
46	No. 11 & 12 (2)	4.2	125	126	50.0	2,000