Influence of Manganese on Growth of Jack Pine and Black Spruce Seedlings

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Résumé

Nos analyses d'aiguilles de pin gris et d'épinette noire, prises dans des peuplements naturels, corroborent les résultats d'observations antérieures: les aiguilles de l'épinette noire contiennent plus de manganèse que celles du pin gris; la teneur en fer est moindre chez l'épinette noire que chez le pin gris.

En chambre contrôlée, on a cultivé des semis d'épinettes et de pins avec des solutions de Mn titrées à 0, 0.1, 1, 10, et 100 p.p.m. C'est dans une solution contenant 0.1 p.p.m. de Mn, que les deux espèces obtinrent leur meilleur développement. On n'a pas observé de différence de tolérance avec l'augmentation de Mn dans les solutions employées.

Aucune différence prononcée, dans la teneur en manganèse des aiguilles, ne fut observée, lors de ces études, au niveau des espèces, comme c'est le cas dans les peuplements naturels. Nous discutons des causes probables de ces faits.

Abstract

Analyses of foliage from natural stands of jack pine and black spruce confirmed earlier observations that levels of Mn were much higher in foliage of black spruce than in that of jack pine. Levels of Fe in jack pine foliage were greater than in black spruce.

In a growth chamber experiment with spruce and pine seedlings and Mn supplied at 0, 0.1, 1, 10 and 100 ppm, both species grew best at the 0.1 ppm level and there was no evidence of difference in tolerance to high Mn levels. In this experiment, no marked species difference in foliar Mn concentrations was observed like that existing in natural stands. Possible reasons for this lack of difference are discussed.

Introduction

In a study on Mn and Fe in eastern Canadian conifers, Gamelin (1937) found that foliage of black spruce (*Picea mariana* (Mill.) BSP) contained higher concentrations of Mn than that of other species, including jack pine (*Pinus banksiana* Lamb.). He noted, too, an association of black spruce with Mn-rich sites and jack pine with sites poorer in this element. Numerous investigators have linked high concentrations of soil Mn with injury to a number of plants, while others have noted species differences in absorption and tolerance. Berger and Gerloff (1947) associated a stem streak necrosis of potato (*Solanum tuberosum* L.) and Adams and Wear (1957) crinkleleaf of cotton (*Gossypium herbaceum* L.) with excess soil Mn. Ross (1961) suggested that some stem cankers in red oak (*Quercus rubra* L.) were related to high soil Mn concentrations. In France, Duchaufour and Rousseau (1960) demonstrated in culture the toxic effect of high concentrations of Mn on silver fir (*Abies alba* Mill.) seedlings. Ingestad (1958) provided data for Norway spruce (*Picea abies* (L.) Karst.) indicating that toxicity symptoms might not be expected until foliar manganese concentration was in excess of 374 parts per million. Below 20 ppm Mn was likely to be deficient.

It has long been observed that following fires in the boreal forest of eastern Canada, some upland sites have regenerated to black spruce, other apparently similar sites have regenerated to jack pine, and yet other sites have varying mixtures of both species. While many factors, including seed supply, seed bed and microclimatic conditions, doubtless have influenced species composition, there exists the possibility that if difference in the tolerance of these two species to Mn exists, and if Mn levels in natural soils cover the appropriate ranges, then forest succession in terms of these two species may be affected.

Materials and Methods

In late August 1963, foliage and raw humus (F & H) samples were taken from 14 stands containing jack pine and black spruce, varying in height from 25 to 55 feet. These stands were located in the Chibougamau area (49°N, 73° 30'W) of Quebec on podsol and podsol-gley soils developed on glacial outwash sands and tills. For large trees, composite samples of current foliage representing the entire crown were taken. For smaller trees, composite samples were taken of current foliage from the mid to lower portion of the crown. These samples were taken to determine generally if the observations of Gamelin (1937), concerning the differences in manganese concentrations in jack pine and black spruce were valid. Manganese in plant and soil samples was determined by the periodate oxidation method (Metson, 1956). The orthophenanthroline method (Jackson, 1958) was used to determine Fe. Exchangeable cations in soils were determined following leaching with neutral normal ammonium acetate solution. Soil pH was determined electrometrically using 1:1 soil water mix.

Two experiments were carried out. The first of these was a preliminary study, in which Mn supply levels were varied from 0.5 to 500 ppm, with levels of other elements held constant. Almost complete mortality amongst seedlings at the highest Mn level after 62 days indicated the desirability of a downward shift in the working range of Mn concentration. A spectrographic analysis on surviving seedlings for P, K, Ca, Mg, Na, B, Fe and Mn showed for increasing Mn supply slight increases in the concentrations of the first six of these elements in both tops and roots, a marked increase in Mn concentration in the roots of both species but a decrease in Fe concentration. Because of the incompleteness of these data (due to mortality) they are not presented here, but this experiment led to a second in which greater control was exercised and in which the apparent Mn-Fe interaction was further investigated.

In the second culture experiment, seeds of jack pine (Seed Zone 2, Ontario Department of Lands and Forests) and black spruce (Seed Zone 3) were pregerminated on moist filter paper, then placed in 10 oz. plastic tumblers filled with perlite, 10 seedlings per tumbler. These tumblers, which had three holes in the bottom, were inserted into light proof plastic trays into which appropriate nutrient solutions (Table 1) were pumped for a three-to four-hour period each day. Five levels of Mn were used: 0, 0.1, 1, 10 and 100 ppm. Mn was supplied as $MnCl_2 \cdot 2H_2O$. Solutions, circulated in four-litre volumes, were changed weekly and the pH adjusted initially to pH 4.5. The Cl concentration ranged from 286 ppm at 0 ppm Mn to 417 ppm at 100 ppm Mn, although over the range 0 to 10 ppm Mn the change in Cl concentration was less than 15 ppm. Thus, the effect of Cl may reasonably be discounted. As a measure of the osmotic pressure the total molar concentrations ranged from 0.012 gram moles per litre at 0 ppm Mn to 0.014 gram moles per litre at 100 ppm Mn, with the change over the range 0 to 10 ppm Mn being negligible. Thus, the osmotic effect

may be discounted also. The experiment was carried out in a growth chamber where the photoperiod was 16 hours; day temperatures were $21 \pm 1^\circ C$ and night temperatures $11 \pm 1^\circ C$. Each treatment was replicated four times. The total growing period was 106 days and total seedling dry weight, top weight, root weight and Mn and Fe concentrations were determined at harvest.

To test if seed germination itself was affected by Mn levels, a small experiment was carried out in which jack pine and black spruce seeds were germinated in contact with solutions in which the Mn concentrations varied from 0 to 1000 ppm. No differences were found related to either Mn levels or species.

Results and Discussion

In natural stands the level of Mn was four to five times as great in black spruce foliage as in that of jack pine (Table 2). There were higher concentrations of Mn in raw humus from under black spruce than in raw humus from under jack pine. Levels of Fe in jack pine foliage were generally greater than in black spruce. No jack pine were present in the black spruce stands but, with one exception, black spruce were present in the jack pine stands. These results substantiate the findings of Gamelin (1937).

In the main experiment, both species attained maximum weight under the 0.1 ppm Mn treatment (Table 3). Statistically ($P = 0.05$), only seedlings of both species at 100 ppm and jack pine at 0 ppm were smaller than those at the 0.1 ppm level. Seedlings of both species at the 100 ppm level had slightly chlorotic foliage. There was no evidence of root necrosis as reported by Duchaufour and Rousseau (1959) for silver fir. Manganese concentration increased markedly in the tops of both species with increase in Mn supply. In roots there was little difference in Mn at the 0 and 0.1 concentrations, but a marked increase at 1, 10 and 100 ppm. Concentrations of Mn in roots of black spruce at these levels of supply were approximately twice that in jack pine roots. Fe levels in the tops of both species were similar and varied little with Mn supply: Fe concentrations in roots were greatest at the lowest level of Mn supply and were much greater in jack pine than in black spruce.

Table 1.
Consumption of nutrient solution supplied to seedlings.

Source	Element Supplied	Concentration
		ppm
NH_4NO_3	Nitrogen	112
KH_2PO_4	Potassium	78
	Phosphorus	62
$CaCl_2 \cdot 2H_2O$	Calcium	161
$MgSO_4 \cdot 7H_2O$	Magnesium	48
	Sulphur	64
$FeC_6H_5O_7 \cdot 7H_2O$	Iron	2.84
H_3BO_4	Boron	0.42
$ZnSO_4 \cdot 7H_2O$	Zinc	0.05
MoO_3	Molybdenum	0.03
$CuSO_4 \cdot 5H_2O$	Copper	0.02
$MnCl_2 \cdot 4H_2O$	Manganese	0, 0.1, 1, 10, 100
	Chlorine	286, 286, 287, 287, 299, 417.

The differences in foliage Mn levels in natural stands may well reflect not a species difference **per se** but rather the fact that black spruce has a much shallower root system than jack pine. As a consequence, a greater proportion of the absorbing roots of the spruce are in the upper organic layers of the soil wherein occurs the highest concentration and the most rapid turnover of Mn. In the experiment, no such variation in root distribution occurred and similar levels of Mn in the tops of both species might be expected and were, in fact, found. Thus, it can be seen that while Mn toxicity as a phenomenon can be clearly demonstrated, it does not seem that species differences in Mn tolerance, at least at the seedling stage of development, occur. Both species showed maximum dry weight development at 0.1 ppm Mn and at increasing levels dry weight was reduced.

The nature of the inhibitory action of excess Mn on plant growth has been the subject of much investigation. Often too, excess Mn has been associated with symptoms similar to those of Fe de-

ficiency. The toxicity of Mn may be related to one or more of the following three general mechanisms.

1. Excess Mn in the tissues is metabolically toxic to one or more of the vital processes.
2. Excess Mn may inactivate or substitute for Fe, possibly precipitating the Fe or the organic reactant with which it was to combine. Somers and Shive (1942) proposed that if Fe is absorbed in the ferric state it is reduced to the ferrous state within the plant, but that excess Mn will cause its re-oxidation to the ferric state and subsequent precipitation as ferric-phosphorus organic complexes. Sideris and Young (1949) working with pineapple (*Ananas comosus* (L.) Merr.) concluded that the toxic effect of Mn resulted from its substitution for Fe in a precursor of chlorophyll. Hewitt (1954) advanced a similar view.
3. Excess Mn prevents entry of Fe into the plant or the transport of Fe, if absorbed, from the roots to other parts of the plant. Twyman (1951) considered the absorbing process to be the limiting one in which Mn competes with Fe for absorp-

Table 2.

Manganese and iron concentrations and pH values for various natural stands of jack pine and black spruce in Quebec.

Stand Type	pH	raw humus		foliage			
		exchange		jack pine		spruce	
		Mn	Fe	Mn	Fe	Mn	Fe
		ppm		ppm		ppm	
Jack pine, young.....	3.6	30	5	150	73	770	40
Jack pine, young.....	4.1	60	3	100	137	—	—
Jack pine, mature.....	3.7	70	8	170	179	690	35
Jack pine, mature.....	3.9	130	23	180	166	640	72
Jack pine, mature.....	4.1	60	3	170	232	750	104
Jack pine, mature.....	4.0	60	11	220	262	870	63
Black spruce, young.....	4.0	220	35	—	—	630	118
Black spruce, young.....	4.0	230	25	—	—	690	96
Black spruce, mature.....	3.4	130	18	—	—	660	72
Black spruce, mature.....	3.4	40	5	—	—	610	83
Black spruce, mature.....	3.8	110	10	—	—	760	86
Black spruce, mature.....	4.0	200	11	—	—	600	125
Burned cutover.....	3.9	100	5	100	221	170	52
Burned cutover.....	4.5	160	11	120	141	870	124

Table 3

Seedling dry weights and manganese and iron concentrations.

Species	Mn Supply ppm	Dry weight			Manganese		Iron	
		top	root	total	top	root	top	root
		grams			ppm		ppm	
Jack pine.....	0	362	100	462	10	32	53	287
	0.1	545	155	700	33	24	51	164
	1	451	128	579	202	47	49	203
	10	458	138	596	855	380	52	164
	100	322	96	418	4445	1731	64	164
Black spruce.....	0	219	26	245	27	35	60	90
	0.1	273	36	309	45	33	68	82
	1	236	28	264	167	85	56	51
	10	261	34	295	1028	674	56	70
	100	171	25	196	4256	3473	62	63

tion sites or primary acceptors involved in the absorption of these ions. Smith and Specht (1953) working with seedlings of citrus trees observed that Mn did induce an Fe deficiency chlorosis and concluded that it did this by interfering with the upward transport of Fe since Fe accumulated in the roots.

In the present study, no attempt was made to ascertain the mechanism of toxicity. Although the amount of Mn within the seedlings increased greatly with increase in external Mn and the Fe concentra-

tion decreased, the magnitude of the Fe decrease did not seem sufficient to explain the entire growth reduction in terms of a simple prevention of Fe absorption alone. There is no indication that increased Mn supply has resulted in a reduced transport of Fe from the roots to the tops. Excess Mn may have prevented chlorophyll production to a degree or in some way inactivated the absorbed Fe; thus, seedlings at the 100 ppm Mn supply were slightly chlorotic. There remains the possibility that the toxicity effect of excess Mn on growth may be quite independent of Fe metabolism.

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From Nova Scotia's Historic shores and storied green hills, the Department of Lands and Forests extends best wishes to the Canadian Institute of Forestry on the occasion of its Diamond Jubilee.

The sharpened use of Canada's forest resources makes the 1968 theme — "New Forests for Old" — especially topical at St. John's, in September.

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