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RESPONSE OF ECTOMYCORRHIZAL *QUERCUS RUBRA* TO SOIL CADMIUM, NICKEL AND LEAD

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Summary—The role of ectomycorrhizae in the uptake of heavy metals by *Quercus rubra* was examined. *Quercus rubra* seedlings inoculated with the ectomycorrhizal fungus *Suillus luteus* were grown in a sandy loam soil containing a range of Cd, Ni or Pb concentrations. Ectomycorrhizal colonization rates were significantly reduced on *Quercus* seedlings by the heavy metals, particularly Cd and Ni. At high soil-heavy-metal concentrations leaf Cd, Ni and Pb content were lower in ectomycorrhizal seedlings compared to nonmycorrhizal controls. Root heavy-metal contents were also higher in ectomycorrhizal plants. Noninoculated seedlings exhibited macro- and microelement deficiencies. The size of nonmycorrhizal seedlings exposed to heavy metals was reduced compared to those inoculated with *S. luteus*. Ectomycorrhizal colonization and growth of *Quercus* seedlings are influenced by high soil concentrations of Cd, Ni or Pb.

INTRODUCTION

The consequences of heavy-metal pollution on forest ectosystems can be widespread and long-lasting (Burton *et al.*, 1983a; Kelley *et al.*, 1979). Of particular concern are the effects on establishment and growth of tree seedlings which may be especially sensitive to stress from soil and air pollution (Berry, 1982; Berry and Marx, 1976). Severe contamination by pollutants, such as heavy metals, can result in widespread seedling mortality and delay revegetation for decades (Ritchie and Thingvold, 1985). Reductions in dry matter production of broadleaf and conifer species have been attributed to soil containing heavy metals such as Cu, Pb and Zn (Smith and Brennan, 1984; Burton *et al.*, 1983a).

The mechanisms of heavy-metal uptake by mature or juvenile woody plants are poorly understood (Smith and Brennan, 1984; Antonovics *et al.*, 1971). Essential heavy metals, such as Cu and Zn, move into plant cells by metabolic pathways (Tinker, 1981). However, the nonessential elements, such as Cd and Pb, may enter cells by metabolic or nonmetabolic mechanisms (Lee *et al.*, 1976). The entry of toxic ions into cells may also take place by competition with essential ions of similar ionic radii (Tinker, 1981).

The threshold level of metal toxicity in woody plants can be highly variable (Burton *et al.*, 1983b; Antonovics *et al.*, 1971). For example, the concentration of Cd in tissue that causes a decrease in the growth of *Acer* and *Quercus* is variable (Smith and Brennan, 1984; Russo and Brennan, 1979). Processes to prevent phytotoxicity include the formation of insoluble crystals, the formation of vesicles, the retention of elements by cell walls and their dilution into nonvital organs (Tinker, 1981; Lee *et al.*, 1976; Antonivics *et al.*, 1971). Tolerance to heavy metals may be significantly influenced by accumulation of toxic ions in root cells (Tinker, 1981).

Field investigations have revealed that ectomycorrhizal fungi may influence seedling absorption of and tolerance to selected heavy metals in soils (Berry, 1982; Berry and Marx, 1976). Seedlings with abundant ectomycorrhizae grow rapidly in toxic strip mine overburden (Berry, 1982). The mechanism by which ectomycorrhizae reduce seedling toxicity to heavy metals may be associated with fungal mantle protection of roots or modification of the rhizosphere by the fungal associate. However, heavy metals can be fungitoxic and may influence ectomycorrhizal formation (Ross, 1982; Ashida, 1965). Bowen (1973) stated the uptake of trace elements or their metabolism by ectomycorrhizal fungi has received little attention.

Several investigations have indicated there is variability in the effect of mycorrhizal symbiosis on host metal uptake and tolerance. Ericoid mycorrhizal associations can protect the host from Cu and Zn toxicity (Bradley *et al.*, 1981). The role of vesicular–arbuscular mycorrhizas (VAM) appears to vary with host and fungal isolate. Gildon and Tinker (1983) reported that VAM associations protect the host from metal toxicity. In contrast, Killham and Firestone (1983) reported VAM increases the uptake of toxic metals, thereby decreasing growth. Ectomycorrhizal associations may enhance host tolerance to Ni, Cu and Zn (Jones and Hutchinson, 1986; Mitchell *et al.*, 1984; Bowen *et al.*, 1974). Mitchell *et al.* (1984) revealed that species of ectomycorrhizal fungi influence essential element content of *Quercus* leaf tissue (Mitchell *et al.*, 1984). The role of ectomycorrhizae in the uptake of nonessential elements and host tolerance to toxic metals is a complex relationship which deserves further investigation.

My objective was to determine the influence of the ectomycorrhizal fungus *Suillus luteus* (L.: Fr.) on the growth and element uptake of *Q. rubra* L. seedlings subjected to a range of concentrations of Cd, Ni or Pb applied to soil.

MATERIALS AND METHODS

Q. rubra acorns were collected from a single tree in Ramsey County, Minn., U.S.A. Following stratifi-

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Table 1. Total and extractable concentrations of Cd, Ni and Pb in a sandy loam soil

Concentration added ($\mu g\ g^{-1}$)		Extractable concentration ($\mu g\ g^{-1}$)
Cd	0	0
	5	2
	10	5
	20	12
	50	26
	100	32
Pb	0	0
	5	1
	10	2
	20	9
	50	21
	100	34
Ni	0	0
	5	2
	10	6
	20	12
	50	31
	100	48

cation and germination, *Quercus* seedlings were transplanted into the potting media.

Seedlings were grown in a sandy loam soil containing approx. 20, 32, 70, 65 and 40 $\mu g\ g^{-1}$ of available [NO_3-N], P, K, Ca and Mg, respectively (Bildusas *et al.*, 1986). The soil solution pH was 6.0 and its organic matter content was 1.5% The soil was sterilized with methyl bromide. Soil physical and chemical properties were characterized by techniques described by Dixon and Buschena (1987) and Chapman and Pratt (1961).

Plastic pots (15 × 15 cm) were filled with sterile soil. One-half of the pots were inoculated with the ectomycorrhizal fungus *S. luteus* using procedures described by Danielson *et al.* (1984). The source and the history of this inoculum is described by Dixon and Buschena (1988). The chloride salt of Cd, Ni and Pb was added singly, in solution, to yield soil solution concentrations listed in Table 1. The procedures described by Burton *et al.* (1983b) were used to implement the soil treatments. A factorial experimental design of four metal treatments (three metals plus control) × two ectomycorrhizal treatments (*S. luteus* inoculated and noninoculated) × eight seedling replicates was utilized.

The *Quercus* seedlings were grown in a glasshouse with diurnal ambient temperatures of 25 ± 3.5°C, relative humidity of 50 ± 5%, and quantum flux density of approx. 250 mol $m^{-2}\ s^{-1}$ during a 16 h photoperiod. A half-strength Hoagland's solution was added to each pot during the 8th wk of the study (Hoagland and Arnon, 1939). The soil–water potential was maintained near −0.1 MPa using gravimetric methods.

Seedlings were harvested 16 wk after transplanting. Whole seedlings were carefully extracted from the soil and separated into roots and shoots. Leaf and root tissue were gently washed using the techniques of Smith and Storey (1976). The number of lateral roots, percentage ectomycorrhizal infection, root and shoot dry wt (72°C, 48 h) were determined. Ectomycorrhizal infection was quantified using the methods of Mitchell *et al.* (1984). Soil and tissue elements were extracted using the methods of Chapman and Pratt (1961) and analyzed by inductively-coupled plasma spectroscopy (Barnes, 1978). Student's *t*-tests were used for statistical analysis of data.

RESULTS

The addition of the heavy metals to the potting media significantly decreased ectomycorrhizal infection by *S. luteus* in association with *Q. rubra* (Table 2). Levels of Cd and Ni above 200 $\mu g\ g^{-1}$ prevented ectomycorrhizal formation on seedling root systems. The presence of Pb above 50 $\mu g\ g^{-1}$ also significantly reduced ectomycorrhizal formation. Ectomycorrhizae were not observed on the noninoculated seedlings and the integrity of the controls was maintained.

The total dry weights and leaf areas of the ectomycorrhizal *Q. rubra* seedlings were 53 and 35% greater, respectively, than the noninoculated controls (Table 2). The addition of 10 or 20 $\mu g\ g^{-1}$ Cd, Ni or

Table 2. Growth and ectomycorrhizal development of *Q. rubra* seedlings grown in Cd, Ni or Pb amended sandy loam soil after 16 wk

Growth variable	Element	*Suillus luteus* inoculation	Concentration added ($\mu g\ g^{-1}$) 0	5	10	20	50	100
Total dry wt (g)	Cd	+	13.2a*	11.6a	11.8a*	10.6a*	5.9b	2.6c
		−	8.6a	8.8a	8.5ab	6.2b	3.4c	2.0c
	Ni	+	13.2a*	12.9a*	11.1abc*	11.2abc*	9.3bc*	8.5c
		−	8.6ab*	9.0a	8.5ab	8.4ab	6.6b	6.9b
	Pb	+	13.2a*	13.2a*	12.7a*	13.1a*	9.8b	10.4b*
		−	8.6a	8.7a	8.2a	8.0a	7.8a	6.7a
Total leaf area (cm^2)	Cd	+	381a*	390a*	324b*	319b*	152c*	64d
		−	282a	277a	201b	125c	63c	48c
	Ni	+	381a*	403a*	392a*	367a*	164b*	71c
		−	282a	308a	276a	112b	53bc	34c
	Pb	+	381a*	392a*	398a*	375a*	227b	230b*
		−	282a	291a	285a	290a	201b	62c
Ectomycorrhizal laterals (%)	Cd	+	67a*	64a*	12b*	3b	0	0
		−	0	0	0	0	0	0
	Ni	+	67*	51a*	19b*	16b*	0	0
		−	0	0	0	0	0	0
	Pb	+	67*	60a*	57a*	64a*	39b*	28b*
		−	0	0	0	0	0	0

Within a seedling variable, means followed by a common letter do not differ at the 5% level.
Within a seedling variable, asterisk (*) indicates ectomycorrhizal inoculation effect is significantly different at the 5% level.

Table 3. Element content in leaves and lateral roots of ectomycorrhizal and nonmycorrhizal *Q. rubra* seedlings grown in Cd amended sandy loam soil after 16 wk

		Concentration added (μg Cd g^{-1})					
		0		20		100	
Element	*S. luteus* inoculation	Leaves	Roots	Leaves	Roots	Leaves	Roots
P (mg)	+	2.9*	2.4abc*	2.8ab*	2.4abc*	2.3bc	2.2c
	−	2.0a	1.9a	2.1a	1.8a	2.2a	1.9a
Mg (mg)	+	18.5a*	16.3ab	17.9a	16.0b	16.4ab	14.8b
	−	14.1abc	12.8bcd	15.2ab	11.7cd	16.2a	10.5d
Cd (μg)	+	1c	2c	2c	8c	35b*	87a*
	−	3b	2b	5b	9b	61a	58a
Zn (μg)	+	31b	45a	28bc	31b	32b	20c
	−	19ab	28a	16b	27a	11b	14b
Fe (μg)	+	216a	164b*	205ab*	173ab*	194ab*	104c*
	−	190a	102b	168a	73bc	96b	46c

Within an element, means followed by a common letter do not differ at the 5% level.
Within a seedling variable, asterisk (*) indicates ectomycorrhizal inoculation effect is significantly different at the 5% level.

Pb significantly reduced the dry weights and leaf areas of noninoculated seedlings relative to the inoculated plants. Significant growth reductions of the ectomycorrhizal seedlings receiving heavy metals were not apparent until 50 μg g^{-1} of Cd, Ni or Pb had been added to the soil. The reductions in total dry wt associated with the Pb treatment were smaller than those observed with the Cd or Ni treatments.

The distribution of Cd, Zn, Fe, Mg and P in the *Quercus* seedlings was significantly influenced by ectomycorrhizal colonization and the amount of Cd added to the soil (Table 3). Inoculation with *S. luteus* significantly increased the content of P, Mg, Zn and Fe in roots and leaves relative to the noninoculated control seedlings. In contrast, ectomycorrhizal colonization decreased leaf Cd content and increased root Cd content of plants growing in soils receiving 100 μg Cd g^{-1}. Leaf Zn and Fe contents decreased in noninoculated seedlings receiving 100 μg Cd g^{-1}. Significant amounts of Cd in roots or leaves in either inoculation treatment was not observed below 50 μg Cd g^{-1}. The dry wt of the noninoculated seedlings was negatively correlated with leaf Cd content ($r = -0.83$).

Inoculation with *S. luteus* and the addition of Ni to the soil significantly influenced the Ni, Zn, Fe, Mg and P contents in the *Q. rubra* seedlings (Table 4). Leaf Fe, Zn, Mg and P content were significantly

Table 4. Element content in leaves and roots of ectomycorrhizal and nonmycorrhizal *Q. rubra* seedlings grown in Ni amended sandy loam soil after 16 wk

		Concentration added (μg Ni g^{-1})					
		0		20		100	
Element	*S. luteus* inoculation	Leaves	Roots	Leaves	Roots	Leaves	Roots
P (mg)	+	2.8a*	2.2b	2.9a*	2.2b	2.8a	2.1b
	−	2.1a	1.8a	2.1a	1.9a	2.0a	2.0a
Mg (mg)	+	18.7a*	16.4a*	19.2a*	17.5*	18.9a	17.3a
	−	13.9a	12.7a	14.7a	13.2a	15.2a	13.9a
Ni (μg)	+	1c	2c	3c	16c	25b*	78a*
	−	1b	1b	12b	15b	56a	48a
Zn (μg)	+	29a	38a	30a*	36a*	32a	39a
	−	21b	30ab	20b	35a	21b	32ab
Fe (μg)	+	217a*	151c*	222a	162bc*	211ab*	147c*
	−	188a	99bc	176a	90c	156ab	83c

Within an element, means followed by a common letter do not differ at the 5% level.
Within a seedling variable, asterisk (*) indicates ectomycorrhizal inoculation effect is significantly different at the 5% level.

Table 5. Element concentration in leaves and roots of ectomycorrhizal and nonmycorrhizal *Q. rubra* seedlings grown in Pb amended sandy loam soil after 16 wk

		Concentration added (μg g^{-1})					
		0		20		100	
Element	*S. luteus* inoculation	Leaves	Roots	Leaves	Roots	Leaves	Roots
P (mg)	+	2.8a*	2.4ab	2.7ab*	2.5ab*	2.5ab	2.2b*
	−	1.9ab	2.0ab	2.2ab	2.0ab	2.4a	1.8b
Mg (mg)	+	18.8a*	16.2b*	19.2a*	17.0b*	19.1a	16.8b*
	−	15.2abc	12.7cd	16.1ab	13.3c	17.9a	10.2d
Pb (μg)	+	8b	21a	7b	24a	11ab	21a*
	−	11b	16b	14b	20b	18b	38a
Zn (μg)	+	30bc	43a*	33abc	35ab	36ab*	21c
	−	17b	29a	19ab	31a	16b	19b
Fe (μg)	+	219a*	156c*	210ab*	201ab	203ab	187b
	−	186a	118b	174a	180a	179a	162a

Within an element, means followed by a common letter do not differ at the 5% level.
Within a seedling variable, asterisk (*) indicates ectomycorrhizal inoculation effect is significantly different at the 5% level.

increased in the ectomycorrhizal seedlings relative to the noninoculated controls. Leaf Ni content was lower and root Ni content higher in the ectomycorrhizal seedlings receiving 100 μg Ni g^{-1}. As leaf Ni content increased, leaf Zn and Fe contents decreased in the noninoculated seedlings. The dry weights of the noninoculated seedlings were negatively correlated with leaf Ni content ($r = -0.74$).

The Pb soil treatments and ectomycorrhizal inoculation significantly influenced the distribution of Pb, Zn, Fe, Mg and P in the *Quercus* seedlings (Table 5). Leaf Fe, Mg and P content were significantly greater in the inoculated seedlings relative to the noninoculated controls. In the high Pb soil treatment, root Pb, P and Mg content and leaf Zn content were higher in the ectomycorrhizal seedlings. Seedling dry weights were not significantly correlated with root or leaf Pb content in either inoculation treatment.

DISCUSSION

This study demonstrates the role of ectomycorrhizal fungi in the uptake of heavy metals by *Q. rubra* seedlings. However, *S. luteus* was sensitive to soil heavy-metal concentrations. The ability of *S. luteus* to form ectomycorrhizal structures (e.g. Hartig net or fungal mantle) in association with *Q. rubra* was suppressed by Cd, Pb or Ni. My results confirm the observation that heavy metals may reduce ectomycorrhizal development (Burton *et al.*, 1983b; Jones and Hutchinson, 1986). The precise mechanism of ectomycorrhizal inhibition is not known. The heavy-metal concentrations used in this study are known to be fungitoxic (Ross, 1982; Ashida, 1965; Somers, 1961). Gildon and Tinker (1983) hypothesized that intracellular concentrations of heavy metals in roots may limit the spread of vesicular–arbuscular mycorrhiza (VAM) mycelia. Any comparison of VAM structure and function to ectomycorrhizae must be made with caution. Tolerance to heavy metals may vary widely with the fungal symbiont (Bradley *et al.*, 1981; Jones and Hutchinson, 1986). The possible interactions between metal tolerance in host and symbiont are poorly understood (Harley and Smith, 1983; Killham and Firestone, 1983).

Heavy-metal uptake by plant roots in axenic or hydroponic systems has been studied (Tinker, 1981; Mengel and Kirkby, 1979). Far less is known about the interactions of ectomycorrhizal colonization and heavy-metal supply, particularly at the extreme range of concentrations (i.e. deficiency or toxicity) (Mitchell *et al.*, 1984; Harley and Smith 1983; Bowen, 1973). The influence of ectomycorrhizae is presumed to be greatest for elements which have narrow diffusion zones around the plant root such as Cd, Ni or Pb (Harley and Smith, 1983; Mengel and Kirkby, 1979). In my study, the differential uptake of heavy metals at low and intermediate soil concentrations may be associated with the development of the fungal mantle (Mitchell *et al.*, 1984; Harley and Smith, 1983), binding of metals to roots or fungal structures (Bradley *et al.*, 1981), or well-known interactions between heavy metals and anions (Tinker, 1981). The relatively high tissue contents of macroelements and low amounts of Cd, Ni and Pb in the ectomycorrhizal seedlings support each of these hypotheses.

At high concentrations of soil Cd, Ni or Pb, ectomycorrhizal colonization was reduced and the heavy-metal content of inoculated seedlings was similar to that of nonmycorrhizal seedlings. In contrast, the heavy-metal content was lower in seedlings with abundant ectomycorrhizae. This observation supports the hypothesis of binding of heavy metals by root–hyphal complexes (Bradley *et al.*, 1981). The tolerance of agronomic crops to toxic ions is associated with their ability to form complexes of heavy metals with their root-cell walls (Tinker, 1981). If heavy-metal supply exceeds the quantity of binding sites on cell walls, the metal will penetrate into the protoplasm (Lee *et al.*, 1976). It is possible that heavy metals may bind to root or fungal structures in the sandy loam soil utilized in this study (Killham and Firestone, 1983). The reduced content of heavy metals in ectomycorrhizal seedlings may also be attributable to the precipitation of metal oxalates in the fungi or their seedling host (Gadd and Griffiths, 1978), or a possible restriction of apoplastic transport by a well-developed Casparian strip (Mengel and Kirkby, 1979).

The total dry weights of the noninoculated seedlings were inversely correlated with leaf Cd or Ni contents. Moreover, the Cd, Ni or Pb content of leaves significantly increased and the root content significantly decreased in the noninoculated plants. This observation implies heavy metals were transported from root-to-shoot in the nonmycorrhizal seedlings. The tissue contents of heavy metals which resulted in a growth reduction in the noninoculated seedlings were greater than the contents previously determined for growth reductions in *Quercus* and *Acer* seedlings of similar size and age (Smith and Brennan, 1984; Russo and Brennan, 1979). Seedling tissue Zn and Fe contents were low in nonmycorrhizal seedlings at the highest heavy-metal dose and this deficiency may have contributed to growth reductions (Tinker, 1981; Mengel and Kirkby, 1979).

Growth reductions were not correlated with tissue Cd, Ni or Pb content in the ectomycorrhizal seedlings. Plants inoculated with *S. luteus* generally had greater macro- and microelement contents than the noninoculated seedlings across the range of metal treatments. Metal toxicity in VAM seedlings may be decreased by improved P nutrition (Gildon and Tinker, 1983). Phosphorus uptake is enhanced in ectomycorrhizal *Quercus* (Mitchell *et al.*, 1984) and elevated amounts of P in the rhizosphere may decrease uptake of microelements such as Zn and Cu (Lambert, 1982). The ectomycorrhizal seedlings investigated in this study did not exhibit the Fe and Zn deficiencies that were observed in the noninoculated seedlings. Ectomycorrhizae play a significant role in the uptake of essential microelements in *Quercus* seedlings (Mitchell *et al.*, 1984).

The uptake and translocation of heavy metals in my study significantly influenced the shoot and root Zn and Fe content in the nonmycorrhizal seedlings. There is published evidence that Cd and other heavy metals influence the translocation of essential microelements such as Fe (Smith and Brennan, 1984) and can result in microelement deficiencies in the plants (Tinker, 1981). Cd, Ni or Pb are considered extremely toxic metals in plants, causing respiratory, photo-

synthetic and structural disorders at relatively low concentrations (Tinker, 1981; Foy *et al.*, 1978). Ectomycorrhizae, which depend upon the flow of photosynthate from leaves, may be particularly sensitive to changes in plant C partitioning (Harley and Smith, 1983).

The heavy-metal applications utilized in my study are representative of industrial effluents in Europe and North America (Ritchie and Thingvold, 1985; Burton *et al.*, 1983a). The availability of pollutant heavy metals to seedlings will depend on the species, soil conditions and ambient environment (Killham and Firestone, 1983). My study suggests heavy metals will decrease the ectomycorrhizal development of *Querus* seedlings and the subsequent uptake of selected elements.

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