Phytochemistry, Vol. 26, No. 6, pp. 1627–1630, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

EFFECTS OF VANADIUM, NICKEL AND SULPHUR DIOXIDE ON POLAR LIPID BIOSYNTHESIS IN JACK PINE

ABDUL A. KHAN* and SURJIT S. MALHOTRA

Northern Forestry Centre, Canadian Forestry Service, 5320-122 Street, Edmonton, Alberta, Canada T6H 3S5

(*Revised received* 7 *November* 1986)

Key Word Index—*Pinus banksiana*; Pinaceae; jack pine; lipid biosynthesis; sulphur dioxide; vanadium; nickel.

Abstract—Biosynthesis of polar lipids (phospho- and glycolipids) from [1-^{14}C]acetate was observed in mature needles from hydroponically grown jack pine seedlings. Treatment of the seedlings with vanadium (V) or nickel (Ni) produced marked concentration-dependent inhibitions in the biosynthesis of all polar lipids. Nickel appeared to be more inhibitory than V at 10 ppm. Fumigation of seedlings with gaseous SO_2 (0.34 ppm) also resulted in reduced biosynthesis of polar lipids. Combined treatment of plant seedlings with metal (V or Ni) and SO_2 produced inhibitory effects that were very similar to those produced by metal alone; however, SO_2 did produce an additive inhibitory effect at 10 ppm V.

INTRODUCTION

The increased use of fossil fuels in many industrial operations has been a key factor in increased atmospheric levels of trace heavy metals and sulphur dioxide (SO_2). Detectable levels of vanadium (V) and nickel (Ni) have been reported in particulates and precipitation [1, 2] and in certain lichen species [3] near the Oil Sands operations in northeastern Alberta, Canada. As this area is covered with forests, it is important to assess the effects on forest plants of each of these trace metals alone and in combination with SO_2. Several earlier studies from this laboratory have demonstrated the usefulness of biochemical tests for assessing previsible air pollutant injuries to forest plants [4].

At present, there is little information on V- and Ni-related biochemical effects in forest plant species. Earlier investigations on crop plant species, however, have shown that both V and Ni are absorbed by plant roots [5, 6]. Vanadium was found to reduce growth [7, 8] and affect stomatal conductance [9]; however, its specific biochemical effect was attributed to its effect on ATPases [10–13]. In addition, V has been shown to cause marked changes in various enzymes and metabolites in various plants [7, 14]. Similarly, Ni has been shown to affect various biochemical and physiological processes in crop species [15–18].

In the field, plants are generally exposed to a mixture of pollutants, and biochemical response may be greatly influenced by the types and concentrations of various pollutants in the mixture. In the present study we have investigated the effects of V and Ni, separately and in combination with SO_2, on polar lipid biosynthesis in hydroponically grown jack pine. The study was carried out under controlled conditions in order to determine the biochemical response to V or Ni individually or in combination with SO_2.

*Present address: Alberta Environmental Centre, Postal bag 40000 Vegreville, Alberta, Canada T0B 4L0.

RESULTS AND DISCUSSION

Polar lipid biosynthesis from [1-^{14}C]*acetate in needles of hydroponically grown jack pine*

In mature needles of hydroponically grown jack pine, [1-^{14}C]acetate was incorporated into various fractions of polar lipids (Table 1). About 60% of the label was in the phospholipids and 40% in the glycolipids. Within phospholipids, the incorporation was highest in phosphatidyl choline (PC), followed closely by phosphatidyl glycerol (PG) and then phosphatidyl ethanolamine (PE) and phosphatidyl inositol (PI). In the glycolipid fraction the incorporation was highest in monogalactosyl diglyceride (MGDG), followed by digalactosyl diglyceride (DGDG) and esterified sterol glycoside (ESG).

The biosynthesis of polar lipids in the mature needles of

Table 1. Biosynthesis of polar lipids from [1-^{14}C]acetate in mature needles of hydroponically grown jack pine

Lipid fraction	Incorporation* 10^{-4} cpm/hr/g dry wt)	% of total polar lipids
Phospholipids		
PC	20.1 ± 0.4	23.9
PG	18.2 ± 0.5	21.6
PE	10.5 ± 0.2	12.5
PI	3.0 ± 0.2	3.6
Glycolipids		
MGDG	14.1 ± 0.2	16.8
DGDG	9.8 ± 0.4	11.7
ESG	8.3 ± 0.2	9.9

PC—Phosphatidyl choline; PG—phosphatidyl glycerol; PE—phosphatidyl ethanolamine; PI—phosphatidyl inositol; MGDG—monogalactosyl diglyceride; DGDG—digalactosyl diglyceride; ESG—esterified sterol glycoside.

*Mean and s.d. ($n = 6$).

hydroponically grown jack pine show a slightly different pattern from that reported earlier in developing needles of seedlings grown in peat moss [19]. A major difference was a marked decrease in PE biosynthesis in mature needles. A similar reduction in PE biosynthesis was reported in lodgepole pine during maturation of needles [19]. It would appear that such changes in the biosynthetic components are probably linked with specific developmental needs in the tissues. For example, marked increases in chlorophyll and chloroplast lipids (MGDG, DGDG and sulphoquinovosyl diglyceride) had been reported during maturation of lodgepole pine needles [20]. One cannot, however, overlook the differences in growth conditions that may influence the biosynthetic pattern.

Effects of V *and* SO_2 *on polar lipid biosynthesis*

Treatment of seedlings with V for 7 days caused a marked inhibition, which was concentration dependent, in the biosynthesis of all polar lipids (Table 2). At a V concentration (10 ppm) producing no visible needle injury, the biosynthesis of polar lipids was inhibited noticeably. The effects were especially pronounced in PG and MGDG biosynthesis, two of the glycerolipids that are characteristic of chloroplast membranes [21]. At higher V concentrations (50 and 100 ppm), biosynthesis of all polar lipids was severely reduced, which indicates that the inhibitory effects were not specific. Such effects are possible if V were to interfere with reactions involved in either the synthesis of acyl moieties or their acylations in various polar lipids. High V concentrations also resulted in progressive darkening of root surfaces and increased foliar desiccation and chlorosis; a marked increase in foliar V levels was also observed in these seedlings (Khan, A. A. and Malhotra, S. S., unpublished results). In separate experiments, it was observed that seedlings exposed to V at 50 ppm for 7 days resulted in more inhibition in polar lipid biosynthesis than those exposed for 3 days.

Fumigation of plant seedlings with SO_2 (0.34 ppm for 3 days) also caused a marked decrease in the biosynthesis of all the polar lipids (Tables 2 and 3); however some glycerolipids were inhibited more than others. The magnitude of these effects on the biosynthesis of various lipids in different experiments could be influenced by various biological and physical factors associated with the experimental material. It was, however, observed that plant samples of the same batch behaved quite similarly and variation was minimal and statistically non-significant.

The differential effects of SO_2 on lipid biosynthesis may be due to different cellular sites and sensitivities of various biosynthetic reactions involved in this process [19]. The biosynthesis of polar lipids was also found to be markedly less sensitive to SO_2 in hydroponically grown jack pine seedlings than in plant seedlings grown in peat moss. In addition, the differences in magnitude of SO_2 effects on lipid biosynthesis as compared to those reported earlier [19] were also due to the high air flow fumigation system (100 l./min) used in this study.

In the combination treatment (V + SO_2), the plant seedlings were exposed to V for the first 4 days and then to both V and SO_2 for 3 subsequent days. In this treatment, the inhibitory effects on the polar lipid biosynthesis were additive only at 10 ppm V; at higher V concentrations, however, the effects were not additive and were similar to those observed with V alone (Table 2). The lack of additive effects of SO_2 at high V concentrations could be attributed to initial severe effects induced by V alone. Later studies, however, showed that simultaneous exposure of plant seedlings to V (50 ppm) and SO_2 (0.34 ppm) for 3 days produced inhibitory effects similar to those observed with V alone.

Effects of Ni *and* SO_2 *on polar lipids biosynthesis*

Treatment of pine seedlings with Ni for 7 days also caused a marked concentration-dependent inhibition in the biosynthesis of all polar lipids (Table 3). Nickel at all concentrations appeared to be more inhibitory than V. Although low concentrations of Ni (10 ppm) did not produce visible injuries in the seedlings or cause an increase in foliar Ni concentrations (Khan, A. A., and Malhotra, S. S., unpublished results), these treatments markedly inhibited the biosynthesis of all polar lipids. It is evident from these results that the biochemical reactions

Table 2. Effects of V singly and in combination with SO_2 on polar lipids biosynthesis in needles from jack pine

	V*			V + SO_2			
	10	50	100	0	10	50	100
Lipid fraction	(% of untreated control)						
Phospholipids							
PC	75.5	25.5	6.0	83.0	47.7	21.4	11.6
PG	51.8	31.1	8.1	85.7	42.8	24.9	9.8
PE	93.0	41.7	9.6	66.8	80.3	27.0	13.5
PI	86.7	37.9	10.6	62.1	47.5	31.3	14.6
Glycolipids							
MGDG	53.9	32.0	5.3	65.9	28.8	16.6	8.7
DGDG	81.9	35.7	8.6	64.8	49.5	25.7	13.8
ESG	71.0	35.5	9.7	71.4	60.2	32.8	13.5

*The numbers under V represent the concentrations of V (ppm) to which the seedlings were exposed through the roots; SO_2 concentration in the fumigation chamber was 0.34 ppm.

Exposure of seedlings to V in both 'V' as well as 'V + SO_2' experiments was for 7 days. 'V + SO_2' seedlings received SO_2 only for the last 3 days of the 7 day period.

Table 3. Effects of Ni singly and in combination with SO_2 on polar lipids biosynthesis in needles from jack pine

Lipid fraction	Ni*			Ni + SO_2			
	10	50	100	0	10	50	100
	(% of untreated control)						
Phospholipids							
PC	34.2	14.5	6.3	70.9	32.0	26.2	9.6
PG	32.8	18.8	6.6	71.2	30.8	29.0	13.7
PE	39.8	22.1	9.6	77.9	41.0	33.1	13.7
PI	37.2	20.7	5.7	63.6	33.0	19.5	10.0
Glycolipids							
MGDG	30.9	11.2	3.9	61.2	34.1	14.3	7.9
DGDG	36.0	11.4	4.2	61.6	30.2	11.6	6.9
ESG	55.7	33.1	8.4	89.9	55.7	32.1	15.0

*The numbers under Ni represent the concentrations of Ni (ppm) to which the seedlings were exposed through the roots; SO_2 concentration in the fumigation chamber was 0.34 ppm.

Exposure of seedlings to Ni in both 'Ni' as well as 'Ni + SO_2' experiments was for 7 days; 'Ni + SO_2' seedlings received SO_2 only for the last 3 days of the 7 day period.

involved in the biosynthesis of polar lipids in pine needles are very sensitive to Ni treatment. At higher Ni concentrations very severe effects on lipid biosynthesis were observed; these concentrations also produced visible phytotoxic injuries on the roots (darkening of surfaces, brittle root hairs) and needles (tip and spotty chlorosis, wilting) and caused marked increases in the Ni concentrations of both tissues (Khan, A. A. and Malhotra, S. S., unpublished results).

Treatment of plant seedlings with Ni and SO_2 together was done in a manner similar to that described for V and SO_2, i.e. the plant seedlings were exposed to Ni for the first 4 days and then to both Ni and SO_2 for the subsequent 3 days. The results showed that a combination treatment of Ni and SO_2 produced inhibitory effects similar to those observed with Ni alone.

To our knowledge, the biochemical changes reported in the preceding sections have not been studied previously in either forest plant or crop plant species. As both phospho- and glycolipids are characteristic constituents of cellular membranes and specialized cellular organelles, the marked effects on the biosynthesis of these lipids due to single or combined pollutant treatments are indicative of biochemical injuries at multiple cellular sites. The mechanism(s) by whilch V and Ni inhibited the biosynthesis of these cellular lipids in pine needles is not clear. It is possible that the effects could be due to interaction of metals with (i) enzyme(s) involved in the biosynthetic process, (ii) intermediary metabolites and (iii) essential and active groups of enzymes. In biological membranes, several forms of V (deca and ortho vanadate) have been reported to stimulate oxidation of NAD(P)H by superoxide radicals [22–25]. Such stimulation could deplete the supply of NAD(P)H, which is essential for fatty acid biosynthesis. Furthermore, V (as vanadate) has been shown to be a potent inhibitor of ATPases from plant [10–13] and animal [26–29] sources. Other metals (e.g. Cd and Ni) could affect enzyme activities by interfering with either essential sulphydryl [30] or carboxylic [31] groups. For example, Ni has been shown to inhibit the *in vitro* activity of nitrate reductase, a sulphydryl-containing enzyme, in *Saline cucubalus* [32]. A very marked inhibition in polar lipids biosynthesis in pine needles could be associated with the inhibitory effect of Ni on essential sulphydryl groups in enzymes associated with fatty acid synthesis and acylations. Earlier studies have shown that sulphydryl groups are essential for lipid biosynthesis in pine needles [19, 33].

EXPERIMENTAL

Plant material and growth conditions. Jack pine (*Pinus banksiana* Lamb) seeds were sown in vermiculite beds and irrigated with diluted (1:3) Hoagland nutrient soln 2 [34]. After 4–6 weeks under greenhouse conditions [35], the seedlings were gently removed from the vermiculite, and the roots were washed with H_2O to remove any adhering vermiculite. The seedlings were then transferred to a hydroponic medium of diluted (1:3) Hoagland nutrient soln 2. The hydroponic medium was continuously aerated and was changed twice each week. The hydroponic growth room was maintained at 20° and 20 klx light intensity (18 hr photoperiod).

Treatment of plants with metals and SO_2. Three- to 4-month-old seedlings of uniform growth were used. Plants were divided into four sets: set 1, control treatment (nutrient soln only); set 2, metal treatment (metal added as a soln to the nutrient soln); set 3, SO_2 treatment (nutrient soln, but later fumigated with gaseous SO_2); and set 4, metal plus SO_2 treatment (metal added as a soln to nutrient soln and later fumigated with gaseous SO_2). Generally 4–8 seedlings were used for each set. Each seedling was kept in a 1-l. plastic container filled with either nutrient soln or nutrient soln containing metal. Solns of V (as ammonium vanadate) and Ni (as nickel acetate) were adjusted to the pH of the nutrient medium (pH 5.5) prior to their addition. All plant seedlings were then placed in 2 identical high air flow (100 l./min) fumigation cuvettes so that seedlings from sets 1 and 2 were in a clean air cuvette and seedlings from sets 3 and 4 were in a cuvette to receive 0.34 ppm SO_2. Both cuvettes were placed in a growth chamber maintained at 20° and 20 klx light intensity (18 hr photoperiod). During the first 4 days all seedlings received clean air; thereafter, seedlings in the SO_2 cuvette were exposed to 0.34 ppm SO_2 for the next 3 days, while the seedlings in the clean air cuvette received clean air. The concn of SO_2 was maintained by a feedback controller and was continuously measured by a

sulphur monitor (Monitor Lab, model 8450). The flow rate was 100 l./min and the humidity inside the cuvettes was maintained at 60–70% by using prehumidified air. Plants were harvested after fumigation, and the mature needles were used for experimental purposes.

Metabolic studies with [1-^{14}C]*acetate.* Excised slices of the needle tissue (0.5 g) were incubated with [1-^{14}C]acetate as described in ref. [19]. Incorporation of the label in various polar lipid fractions was measured after their extraction and separation by TLC as described in ref. [19]. Radioactivity in each lipid fraction on TLC plates was initially scanned with a radioisotope scanner, and then each spot and radioactive peak was matched with authentic standards. The thin-layer plates were scraped, and the silica gel was added to Omnifluor (New England Nuclear) scintillation fluid (0.4% Omnifluor dissolved with 30% EtOH in toluene) and counted in a liquid scintillation spectrometer [19]. Needle dry wt was measured by oven-drying the fresh needles at 80° for 24 hr. All experiments were repeated at least 3 ×, and the trends were found to be reproducible. The data in the tables are taken from representative experiments.

Acknowledgements—We thank P. A. Hurdle for technical assistance. This work was supported in part by funds from the Alberta Oil Sands Environmental Research Programme.

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