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Long-term exposure of Scots pine seedlings to monochloroacetic and trichloroacetic acid: Effects on the needles and growth

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The effects of monochloroacetic acid (MCA) and trichloroacetic acid (TCA) exposures on Scots pine seedlings were studied. The exposures, with two dose levels for TCA and one for MCA, were done simultaneously via the roots and the foliage during two consecutive simulated growing seasons. An increase in potassium concentration in current-year needles exposed to lower TCA dose after the first exposure season, and an increase in the nitrogen concentration, as well as a decrease in the transpiration rate and in the total chloroplast area, were noted in the current-year needles exposed to MCA after the second exposure season and these changes were statistically different from the control. These results may be due to charge compensation and hormonal changes induced by subtoxic levels of TCA and MCA.

Key words: gas exchange, monochloroacetic acid, needle ultrastructure, nutrient concentration, trichloroacetic acid

INTRODUCTION

Trichloroacetic acid (TCA) has been detected in different compartments of the environment, e.g. precipitation, soil water and conifer needles (Renner *et al.* 1990, Plümacher & Renner 1991, Frank *et al.* 1992, 1994, Plümacher & Schröder 1994, Juuti *et al.* 1995, 1996). The origin of TCA in the environment has been proposed to be the atmospheric degradative reactions of short-chain chlorinated hydrocarbons originating from anthropogenic sources (Frank *et al.* 1992, 1994). Most of these chlorocarbons have long atmospheric residence times, which allow them to reach remote areas. There are some recent indications, however, that TCA may also be formed in soil naturally

(Hoekstra & de Leer 1995, Haiber *et al.* 1996). In precipitation, TCA concentrations up to 20 μg l^{-1} have been reported (Plümacher & Renner 1993, Frank *et al.* 1994, Reimann *et al.* 1996).

Much interest has been focused on TCA, which theoretically could have adverse effects on forest trees due to its phytotoxic nature (Ashton & Crafts 1973), since it was previously used commercially as a herbicide. The phytotoxic levels of TCA have been shown to cause, e.g. inhibition of several enzymes, interference with lipid and carbohydrate metabolism, inhibition of plant growth, induction of leaf chlorosis and production of leaf necrosis (Ashton & Crafts 1973). These levels may also alter the character of the surface wax (Macey 1974, Tevini & Steinmuller 1987). TCA concentrations of individual trees vary considerably and tend to increase with the needle age (Frank *et al.* 1994). The concentrations in the three-year-old needles are typically 1–180 μg kg^{-1} in rural forests (Plümacher & Renner 1991, Frank *et al.* 1992, 1994, Plümacher & Schröder 1994, Juuti *et al.* 1996). Concentrations up to 280 μg kg^{-1} have been reported in the needles from the forests near industrial installations (Juuti *et al.* 1995). The occurrence of MCA, also previously used as a herbicide, has also been detected in various environmental samples (Frank *et al.* 1994). However, there is considerably less data about the effects of MCA on plants than about that of TCA.

TCA from soil water is taken into leaves and needles via the transpiration stream (Blanchard 1954, Sutinen *et al.* 1995). Part of TCA may also be taken via needle surface (Sutinen *et al.* 1995), or as lipophilic precursor chlorocarbons, as suggested previously (Frank *et al.* 1992, Plümacher & Schröder 1994). Experiments with conifer seedlings have shown that exposure to fairly high doses of TCA may influence photosynthesis by changing the chloroplast structure (Sutinen *et al.* 1995), and may also cause changes in the needle surface wax structure (Franich & Wells 1980, Schell & Kristen 1992, Sutinen *et al.* 1995). Needle samples collected in Berlin and its surroundings have been reported to exhibit the most eroded wax structure in those needles with the highest TCA concentrations (Plümacher 1995). The effects of long-term exposure to low levels of TCA or MCA on conifers are not known.

The purpose of this study was to investigate the impacts of long-term exposure to TCA on the ultrastructure of mesophyll cells, rates of photosynthesis and transpiration, needle chlorophyll and nutrient concentration and the growth of Scots pine seedlings. Furthermore, a search was made for specific changes in needle fine structure. The exposures lasted for two simulated growing seasons. The aim was to keep the TCA concentrations in the needles representative of the levels observable in the field. In addition, a preliminary study of the effects of MCA on pine seedlings was made with a fairly high dose level.

MATERIALS AND METHODS

Seedling material

Two-year-old potted seedlings of Scots pine (*Pinus sylvestris* L.) of seed orchard origin, grown at a nursery in Suonenjoki, Finland (62°39´N, 27°03´E, altitude 142 m a.s.l.), were used in the experiment. The seedlings were grown and treated according to normal nursery practice, including fertilizations and occasional pesticide treatments. The seedlings were brought inside in September 1993 and kept in a dark store room (temperature – 5°C). A three-week dehardening régime was started in January 1994, during which the temperature was raised from + 5 to + 15°C and the light period from 10 to 15 hours. After the dehardening period, the seedlings were replanted in a sand–peat soil mixture (1:4 v/v) and moved to a greenhouse. The peat had been prefertilized and is the one commonly used in Finnish tree nurseries (Vapo E D1K2, Vapo Corp., Finland). The growing temperature varied between + 25°C (day) and + 15°C (night), and the relative humidity from 90 to 30%, respectively. The natural light in the greenhouse was supplemented with artificial lighting (HQI 400 W lamps) to give a 20-h photoperiod. The photosynthetic photon flux density at the top of the plants was about 250 μmol m^{-2} s^{-1}.

Exposure technique

The seedlings were exposed to TCA or MCA for two simulated growing seasons. The treatments were performed five times a week during the first two weeks of the first exposure season and 13 times during the following 17 weeks (Fig. 1). The exposure frequency was planned in order to achieve TCA levels in the needles comparable with those observed in the field. After the first exposure period, the seedlings were hardened by reducing the temperature from + 15°C to – 5°C and the photoperiod from 20 hours to darkness. Dehardening of the seedlings was performed after two months in the same manner as earlier. For the second expo-

sure season, the seedlings were again moved to the greenhouse, to similar conditions as before, and treated twice a week with the same dose levels as used previously until sampling in the phase of cessation of growth (10 weeks from the beginning of the second exposure season). During the second exposure season the seedlings were fertilized twice. The simulated winter season caused partial yellowing and browning of the needles that had developed during the first exposure season. Thus only the dry weights of these needles were measured, whereas all the other measurements were performed on current-year needles after the second exposure season.

The aqueous chloroacetic acids were applied simultaneously via the roots and needle surface. Four treatments were used: lower (TCA1) and higher (TCA2) doses of TCA, one dose of MCA and water taken from a nearby lake as a control treatment. Each treatment contained 100 seedlings divided into four replicate blocks of 25, randomly placed on tables in the greenhouse. They were irrigated with lake water via vessels placed beneath them. Based on our earlier experience (Sutinen *et al.* 1995), the irrigation frequency was two to three times a week, which was sufficient to prevent drying of the soil in the pots.

The exposure solutions were prepared by diluting TCA (Merck, Germany) or MCA (Fluka, Switzerland) with the same water from a nearby lake that was used for irrigation and for the control treatments. The TCA concentration of the lake water was 0.3 μg l^{-1}. The exposures via the roots took the form of additions of 5 ml of 0.1 mg l^{-1} or 0.2 mg l^{-1} TCA solutions (TCA1 and TCA2 doses, respectively) or 1 mg l^{-1} MCA solution to each pot. The doses were comparable with the total amounts of 1.0 g, 2.0 g and 10 g per hectare calculated for each exposure season for TCA1, TCA2 and MCA, respectively.

The seedlings were also exposed to foliar mist of these compounds inside Teflon chambers (50-μm thick FEP-Teflon®; volume 0.26 m^3) for about 70 minutes. The fumigations with the TCA-solutions, 10 ml of 0.5 mg l^{-1} or 1.0 mg l^{-1} (TCA1 and TCA2 doses, respectively) or 5 mg l^{-1} MCA solution were applied using cleaned (activated charcoal filter, Wilkerson) and dried (watertrap, Wilkerson) pressurized air nebulizers, as in Sutinen *et al.* (1995). The mist was mixed with a fan (diameter 0.08 m) placed at the top of each chamber. Each of the treatments had its own chamber. Because of the effective mixing in the chamber, the dose obtained by each seedling could neither be measured nor calculated exactly. Most of the fumigated TCA and MCA are assumed to be deposited on the needles and the chamber surfaces.

Determination of TCA

Pooled samples of needles from two seedlings were collected for analyses of TCA. MCA was not detected in the needle samples due to the high detection limit of the method used here. Two seedlings were taken from two different blocks per treatment and the pair of blocks was changed every time. Each seedling was sampled only once. Second-year needles were taken 12 times during the first exposure season and the current-year needles were sampled at the end of the exposures in the second season. The needles were handled with plastic gloves, cut with clean scissors and placed in glass vials. The samples were then stored in the cold (< – 20°C) until analysed. They were prepared in two ways. One half of each sample was ultrasonicated in 150 ml of deionized Milli-Q water for four minutes before the preparations in order to remove any of the compound attached on the needle surface and obtain the concentration inside the needles. The second half was prepared as such and showed the sum of the concentrations on the surface and inside the needles.

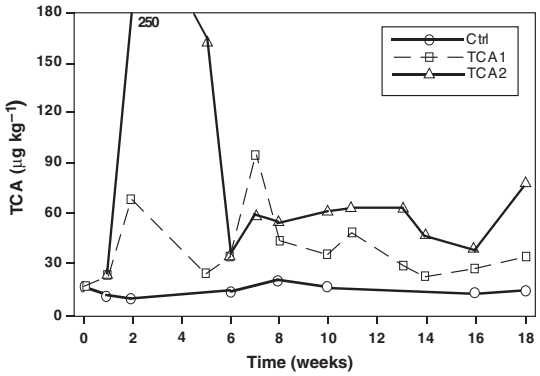

Fig. 1. Trichloroacetic acid (TCA) concentrations in the needles of seedlings exposed to the lower (TCA1) or higher (TCA2) TCA doses applied simultaneously via the roots and foliage, or in the needles of the control seedlings (Ctrl). The seedlings were treated five times during the first two weeks and weekly thereafter, except for weeks 3, 5, 10 and 12. For dose levels, see the text.

The samples were prepared and analyzed as described earlier (Frank *et al.* 1990, Juuti *et al.* 1995). Crushed needles (2 g) were extracted with water (6 ml) and reextracted with ether from the acidified phase. TCA was methylated with diazomethane and analyzed by gas chromatography-mass spectrometry (Hewlett Packard 5890, VG Masslab Trio-2) with negative chemical ionization. An internal standard (2,2-dichloropropionic acid, Aldrich) was used for quantification. Concentrations were expressed on a fresh weight basis.

Chlorophyll concentration and gas exchange

Chlorophyll a and b concentrations in the current-year needles were measured from five seedlings per block of TCA1 and MCA treatments and from seven seedlings per block of TCA2 and control treatments at the end of the second exposure season by the method of Arnon (1949), and gas exchange from the current-year needles of four seedlings per

block at the same point in time, when their growth was completed, with an infrared gas analyzer (Li-6200, Li-Cor Co.). During the one-minute measurements, the CO_2 concentration in the cuvette typically decreased from 355 to 340 μl^{-1}, the cuvette temperature was 23°C, PAR 600 $\mu mol\ m^{-2}\ s^{-1}$ and relative humidity 25%. Gas exchange was measured in a closed system where flow rate was adjusted so that the relative humidity remained stable (air flow was partly through desiccant). Gas exchange was calculated on the basis of the silhouette areas of the seedlings, using a video camera connected to a graphic TV monitor.

Microscopy

Five seedlings from each of the four replicate blocks for each treatment were selected at random for ultrastructural studies at the end of the second growing season. One current-year needle was taken from the upper third of the year's growth on each seedling and was put into a solution containing 1.5% glutaraldehyde, 1.5% paraformaldehyde, cacodylate buffer (pH 7.0, 0.15 M sucrose and 2 mM $CaCl_2$). The molarity of the buffer solution was adjusted to 0.05 M, which is suitable for measurements made during the growing season (Soikkeli 1980). The needles were cut and fixed as described earlier by Soikkeli (1980) and Sutinen *et al.* (1995).

Sections for ultrastructural studies were cut from the mesophyll tissue of each needle and stained with uranyl acetate and led citrate. The mesophyll cells located close to the epidermis of each section and those located near the endodermis were photographed at 4 000 × magnification with a transmission electron microscope. The areas of the chloroplasts, starch grains and cytoplasm were measured by a point counting method (Romppanen & Collan 1984). The number of chloroplasts measured and the number of starch grains per cell were calculated. The total area of chloroplasts and starch grains per 100 μm^2 of cytoplasm was calculated from these figures. Furthermore, the occurrence of abnormalities in fine structure was investigated in each micrograph. The final magnification of the micrographs was 8 000 ×.

Growth and nutrients

The dry weights of the roots and current- and second-year stems and needles were determined after drying them at 80°C for three days at the end of the second exposure season. The number of plants measured varied between 21–37 according to the treatment.

Nutrient concentrations in the current- and second-year needles were measured at the end of the first exposure season and those in the current-year needles at the end of the second season. Two seedlings from each block were sampled, and the needles were dried at 80°C for three days. The same number of needles was taken from each two seedlings of the block and mixed for the measurements. The concentration of N was determined with a LECO CHN-600 analyzer (Leco Co, USA), and those of P, K, Ca, Mg, S, Mn, Fe, Al, Cu and Zn in wet digested HNO_3–H_2O_2-samples (Halonen *et al.* 1983) using plasma emission spectrophotometric analysis (ICP, ARL 3800).

Statistical analysis

Statistical significance was tested with ANOVA followed by Tukey's multiple range test (Systat, Inc., 1992).

RESULTS

Trichloroacetic acid concentrations

The TCA concentration inside the needles was 16 $\mu g\ kg^{-1}$ before the first exposures (Fig. 1). After ten applications during the first two weeks, the levels increased considerably in the needles of the TCA2 treated seedlings, and a clear increase relative to the controls was also noted in the needles of those treated with the lower TCA dose. During weeks 3–6 applications were made only once, and hence the concentrations decreased in both exposure groups. Furthermore, the level in the TCA2 treated needles continued to decrease during weeks 6–8, when there were no exposures. By six weeks after the beginning of the treatments, the TCA concentrations in the C + 1-needles of the TCA1-treated seedlings were about 35 $\mu g\ kg^{-1}$ and those in the needles of the TCA2-treated seedlings about 60 $\mu g\ kg^{-1}$.

By the end of the second exposure season, the TCA concentrations in the newly grown needles (current-year needles) were 4, 17 and 18 $\mu g\ kg^{-1}$ in the control, TCA1- and TCA2-treated seedlings, respectively. About 15–20% of the amount of TCA was observed on the surface of the needles.

Chlorophyll concentration and gas exchange

The total chlorophyll concentration of the current-year needles was higher in the TCA2-treated seedlings than in the other treatments (Fig. 2a). The chlorophyll a:b ratio (Fig. 2b) and net photosynthesis (Fig. 2c) were higher in the TCA1- and MCA-treated seedlings than in the controls or the TCA2-treated seedlings, but the differences were

not statistically significant. The transpiration rate was about 20% lower in all the treated seedlings than in the controls, but the difference was statistically significant (Fig. 2d) only between the MCA-treated and control seedlings.

Ultrastructure

The chloroplasts in the needle mesophyll cells of the seedlings exposed to the TCA1, TCA2 and MCA treatments were 12 and 5% larger and 10% smaller than the controls, respectively, while the mean area of starch grains was 9 and 5% larger and 22% smaller, respectively. The number of chloroplasts decreased 4–7% in all the treated seedlings relative to the controls, but these differences were not statistically significant. The total area of chloroplasts (Fig. 2e) and starch grains (Fig. 2f) per 100 μm^2 of cytoplasm showed similar trends to the mean areas of individual chloroplasts and starch grains. A statistical significance was found only between the MCA-treated and control seedlings in the total area of the chloroplasts (p = 0.040; Fig. 2e). Otherwise the structure of the mesophyll cells was mainly similar to that in healthy conifer needles during a growing season, as described by Soikkeli (1980). Some general stress symptoms, such as an increase in lipids in the cytoplasm and in the number of plastoglobuli in the chloroplasts were noted, as described in our earlier study (Sutinen *et al.* 1995).

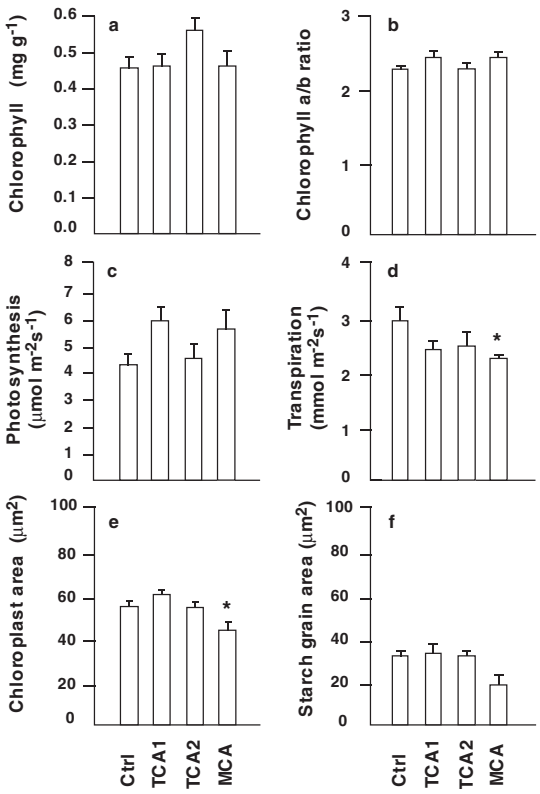

Fig. 2. (a) Total chlorophyll concentration, (b) chlorophyll a:b ratio, (c) net photosynthesis, (d) transpiration rate, (e) total area of chloroplasts and (f) total area of starch grains per 100 μm^2 of cytoplasm in the current-year needles of seedlings exposed to the lower (TCA1) or higher (TCA2) doses of TCA or to MCA, or in the control seedlings (Ctrl). The measurements were performed after the second exposure period. Differences between MCA/TCA exposure groups and the controls: * = p < 0.05.

Growth and nutrients

The dry weights of the needles or stems that had grown during the first exposure season did not show any differences between the treatments (Fig. 3a and c). Instead, the dry weights of the needles that had developed during the second exposure season (Fig. 3b) were 3 and 8% lower in the TCA1- and TCA2-treated seedlings, respectively, and those of the stems (Fig. 3d) 6 and 12% lower. The length growth of the stems during the second exposure season (Fig. 3e) was affected similarly, the stems being 5% shorter in the TCA1-treated seedlings and 12% shorter in the TCA2-treated seedlings than in the controls. The dry weights or the length growth of the above-ground parts of the MCA-treated seedlings did not differ from the control values (Fig. 3a–e). The dry weight of the roots increased by 9, 17 and 14% in the TCA1-, TCA2- and MCA-treated seedlings relative to the controls (Fig. 3f), which has also increased the root:stem ratio of dry weights of these seedlings. The differences in dry weights or in length growth were not statistically significant, however.

The concentrations of macronutrients (N, P, K, Ca, Mg and S) and micronutrients (Fe, Cu, Zn, Mn and Al) in the current-year needles were determined at the end of each exposure season, and those in the second-year needles after the first season. The nutrient concentrations were often higher

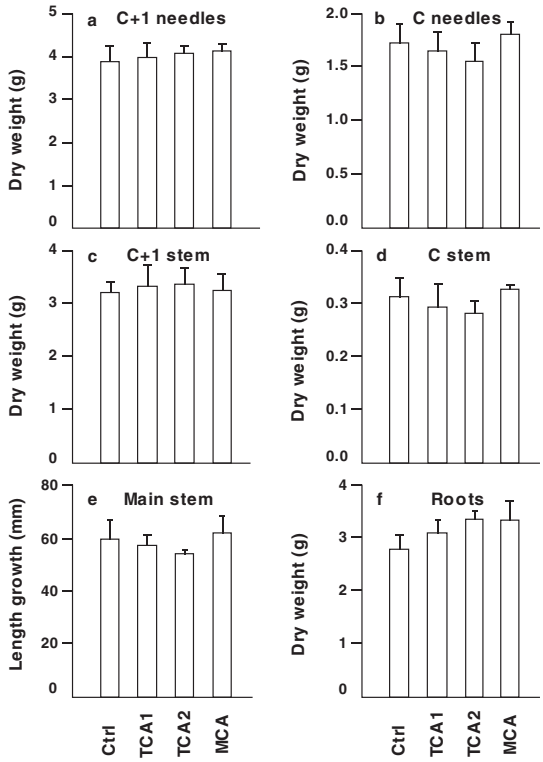

Fig. 3. Dry weight of (a) second-year needles, (b) current-year needles, (c) second-year stems, (d) current-year stems, (e) stem lengths and (f) dry weight of roots in the pine seedlings exposed to the lower (TCA1) or higher (TCA2) doses of TCA or to MCA, or in the control seedlings (Ctrl). The measurements were performed after the second exposure period.

in the treated seedlings than in the controls (Table 1), but the great variation meant that statistical significance was achieved only in the potassium concentration of the current-year needles between the control and TCA1 treatments after the first exposure season, and in the nitrogen concentration between the control and MCA treatments after the second season.

DISCUSSION

The TCA concentrations inside the needles with both TCA doses were within the TCA concentrations found in needles in the field (Frank *et al.* 1994, Plümacher & Schröder 1994, Juuti *et al.* 1995, 1996). The high concentrations in the TCA2-treated needles two to five weeks after the start of the exposures were due to the high frequency of the applications. The fact that the TCA concentration decreased from its peak of 250 μg kg^{-1} to a value of 36 μg kg^{-1} during the following four weeks, when the seedlings were treated only once, demonstrates that TCA can be eliminated from the needles, obviously by metabolism or active transportation (Frank *et al.* 1994). TCA concentrations in needles obviously represent a steady-state between uptake and loss. The exposure frequency used here after the first two weeks (less than once a week) was sufficient to maintain the TCA at the desired levels during the first exposure season.

Even though the exposure frequency was higher during the second exposure season (twice a week), the TCA levels in the current-year needles were lower than those in the second-year needles had been during the first exposure season. It has been noted previously in the field (Frank *et al.* 1992) and experimentally (Sutinen *et al.* 1995) that TCA concentrations increase with needle age. This may be caused by, e.g. dilution of TCA due to growth of needle volume (Sutinen *et al.* 1995) or faster elimination rate of TCA in the younger needles.

The TCA concentrations in the solutions added to the pots containing the seedlings were higher than in soil water in the field (up to 3 μg l^{-1}, Renner *et al.* 1990). It should be noted, however, that the TCA applied to the pots was diluted with the irrigation water that was in each pot, which would result in lower actual exposure concentrations. The TCA concentrations in the misted solutions were much higher than in precipitation in the field (Plümacher & Renner 1993). Since TCA is hydrophilic, its uptake via the needle surface is less important than that via the roots. We noted in our previous experiment that TCA could end up in the needles by both routes, even though most of the misted TCA remained on the needle surfaces (Sutinen *et al.* 1995).

The seedlings exhibited an increase in the potassium concentration in the TCA1-treated needles after the first exposure season compared with the control. Furthermore, in the MCA-treated needles, an increase in the nitrogen concentration and a decrease in the transpiration rate and in the total chloroplast area were noted after the second exposure season. Increases, although not statistically significant, were also found in the chlorophyll a:b

ratio and net photosynthesis in the TCA1- and MCA- treated seedlings, as well as in the root to shoot ratio in all treated seedlings compared with the control. Similar symptoms have been reviewed by Ries (1976) for plants exposed to subtoxic levels of herbicides, i.e. when the amount of herbicides applied to soil have been a few grams per hectare, and the molarities of the solutions 10^{-6} to 10^{-9} M, that are comparable with the molarities of the TCA solutions (1×10^{-6} to 6×10^{-7} M) used in this study. The molarity of the MCA solution was high (10^{-2} M), which was noted to decrease the chloroplast area. This has been observed previously with fairly high TCA doses (Sutinen *et al.* 1995) and suggests interference with photosynthesis.

The increased potassium concentration refers to charge compensation, when the accumulation of CCl_3COO^- anions is counterbalanced with the uptake of K^+ cations into the cytoplasm (Marschner 1995). The changes in the transpiration rate and the increase in the root to shoot ratio may be associated with the hormonal changes due to toxic or subtoxic levels of herbicides (Ries 1976, Morgan 1976, 1990). The increase in the root to shoot ratio may also be induced by slight nutrient deficiency (Marschner 1995). In this study all the nutrient concentrations, except that of nitrogen, which was low, were in the range of those in healthy trees in the field (e.g. Raitio 1990). However, typical changes of nitrogen deficiency in the ultrastructure (Palomäki & Holopainen 1994) were not found. In fact, all the treated seedlings had higher concentrations of nitrogen compared with the control. Increase in nitrogen levels, as well as in several other nutrient concentrations, have been reported earlier in several plant species exposed to low levels of herbicides (Ries 1976).

An accumulation of lipids in the cytoplasm and an increase in the number of plastoglobuli in the chloroplasts of the mesophyll cells were noted in the TCA- and MCA-treated needles. These symptoms have also been reported earlier in TCA-treated conifer needles (Kristen *et al.* 1992, Sutinen *et al.* 1995), and seem to be general signs of stress (Holopainen *et al.* 1992, Sutinen & Koivisto 1995). Thus, the routine microscopic methods used here do not allow the diagnosis of the possible effects of TCA or MCA on needles in the field as they do with some other air pollutants e.g. ozone and sulphur dioxide (Sutinen *et al.* 1990, Holopainen *et al.* 1992). Microscopic investigations of the meristematic regions of both shoots and

Table 1. Nutrient concentrations (mean ± S.D.) in current (C) and second (C + 1)-year needles of pine seedlings exposed to the lower (TCA1) or higher (TCA2) doses of TCA, to MCA or to lake water as a control treatment (Ctrl), measured after the first (Exp. 1) and second (Exp. 2) exposure seasons. For doses, see the text.

Treatment	N %	P mg g^{-1}	K mg g^{-1}	Ca mg g^{-1}	Mg mg g^{-1}	S mg g^{-1}	Fe µg g^{-1}	Cu µg g^{-1}	Zn µg g^{-1}	Mn µg g^{-1}	Al µg g^{-1}
Exp. 1:											
C-needles											
Ctrl	0.76±0.1	1.04±0.2	5.76±0.7	1.56±0.4	1.20±0.2	0.77±0.1	20.4±6.8	1.96±0.4	37.9± 6.5	288±49	48.8± 9.7
TCA1	0.86±0.1	1.12±0.1	7.38±0.4*	1.54±0.3	1.32±0.1	0.84±0.1	26.4±6.7	2.95±1.0	40.6± 2.0	310±22	53.7± 7.0
TCA2	0.80±0.1	1.07±0.1	6.62±0.2	1.58±0.2	1.27±0.1	0.78±0.1	20.7±2.7	2.19±0.3	37.7± 3.6	295±33	45.6± 4.5
MCA	0.73±0.1	1.01±0.1	6.21±0.5	1.36±0.1	1.18±0.1	0.79±0.1	20.1±3.1	2.37±0.3	36.5± 4.4	313±13	48.1±10.0
C + 1-needles											
Ctrl	0.82±0.0	1.18±0.1	6.40±0.7	2.42±0.7	1.15±0.2	0.74±0.0	31.2±4.5	10.4±2.5	46.8± 6.6.	226±32	30.5± 8.0
TCA1	0.81±0.0	1.14±0.0	6.75±0.6	2.46±0.5	1.07±0.2	0.80±0.1	33.6±6.4	10.9±0.7	44.8± 5.0	266±42	36.5±10.3
TCA2	0.82±0.2	1.08±0.2	6.13±0.6	2.51±0.3	1.05±0.1	0.85±0.1	33.2±6.2	13.3±2.6	43.1± 5.8	248±34	32.0± 4.9
MCA	0.76±0.0	1.14±0.2	6.12±0.8	2.18±0.3	0.95±0.1	0.73±0.1	33.5±5.9	9.6±1.0	39.7±11.1	248±28	32.8± 5.2
Exp. 2:											
C-needles											
Ctrl	0.55±0.0	1.10±0.1	9.57±0.6	1.30±0.2	1.17±0.0	0.72±0.0	21.1±1.2	3.43±0.2	31.3± 2.4	317±21	75.3± 6.7
TCA1	0.57±0.0	1.15±0.1	9.61±0.4	1.25±0.1	1.20±0.1	0.76±0.0	20.2±1.7	4.47±1.2	35.4± 5.1	349±54	75.6±16.9
TCA2	0.57±0.0	1.10±0.0	9.53±0.3	1.29±0.1	1.21±0.0	0.72±0.0	23.9±8.7	3.03±0.2	33.9± 1.2	361±15	76.0± 5.2
MCA	0.62±0.0*	1.17±0.1	9.64±0.9	1.35±0.1	1.21±0.0	0.75±0.0	23.0±3.5	4.80±0.8	35.7± 2.0	325±43	76.6± 6.7

ANOVA, Tukey's multiple range test, $p < 0.05$, $n = 4$
* significant difference between control and MCA/TCA treatments

roots, where hormonal regulation occurs, may be more relevant than studies of needle structure, when one wishes to examine the effects of subtoxic levels of herbicides.

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