The routes and kinetics of trichloroacetic acid uptake and elimination in Sitka spruce (Picea sitchensis) saplings via atmospheric deposition pathways

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Abstract

A major flux of trichloroacetic acid (TCA) to forests is via wet deposition, but the transfer of TCA into tree foliage may occur by an above- or below-ground pathway. To investigate the routes and kinetics of TCA uptake, two groups of 10 Sitka spruce saplings (with an equivalent number of controls) were exposed to a single application of 200 mg TCA in solution, either to the soil only, or sprayed as a mist to the foliage only. The needle foliage was subsequently analysed regularly for TCA for 3 months during the growing season. Significant uptake into current year (C) needles was observed from both routes just a few days after application, providing direct evidence of an above-ground uptake route. Uptake of TCA was also observed in the previous year needle class (C + 1). Kinetic modelling of the data indicated that the half-life for within-needle elimination (during the growing season) was ~ 50 ± 30 days. Most of the applied TCA appeared to be degraded before uptake, either in the soil, or externally on the sapling foliage.

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1. Introduction

Elevated concentrations of trichloroacetic acid (TCA) up to 180 ng g\textsuperscript{-1} fwt have been measured in the foliage of forests in many countries (see recent reviews by McCulloch, 2002; Schöler et al., 2003). Since TCA has a herbicidal effect against woody plant species (Barrons and Hummer, 1951), reports of correlations between the TCA content of needles and the extent of defoliation have led to the suggestion of a cause-effect link (Frank, 1991; Norokorpi and Frank, 1995).

The origin of TCA in needle foliage is not entirely obvious. Since TCA is extremely soluble, with a Henry’s law coefficient of the order of 10\textsuperscript{5} M atm\textsuperscript{-1} (Bowden et al., 1998), it will partition almost exclusively into the aqueous phase. Fluxes of TCA from the atmosphere in precipitation significantly exceed estimates of its production via currently understood photooxidation reactions of chlorinated solvents emitted to the troposphere (McCulloch, 2002). Furthermore, an issue to address is whether TCA in needles arises by direct “above-ground” uptake from the atmospheric aqueous phase (via needles or branchwood), or indirect uptake from the soil via the vascular system. The former may be important since many coniferous forests are in zones of prolonged contact with convective cloud even if actual wet precipitation is low. Quantification of the rates of uptake and elimination under controlled conditions will improve understanding of the cycling of TCA through the forest system, and is important for the risk
assessments of TCA and its precursors imposed on manufacturers of relevant chlorinated compounds.

In this work, Sitka spruce saplings were pulsed with a single dose of TCA solution to soil or foliage only, during a growing season, to follow the kinetics of uptake into needle foliage and subsequent elimination. Previous similar studies have not investigated uptake routes in Sitka spruce and have generally used multiple doses of TCA applied to considerably younger and smaller seedlings. This study used six-year old saplings which are likely to have up to 100 times the volume of plant material giving 100 times lower dose per plant. Sitka spruce is the major commercial forest species in the UK (Mason and Sharpe, 1992).

2. Materials and methods

The 40 six-year old Sitka spruce saplings (Picea sitchensis (Bong.) Carr.) of Queen Charlotte Island provenance, used in this experiment were grown in pots containing a 3:1:1 peat:loam:grit mixture in an unheated greenhouse. The mean (±s.d.) height and root collar diameter of the saplings at the start of the growing season (April 2002) were 105±12 and 2.3±0.2 cm, respectively. The saplings were systematically divided into four groups each containing one of the four tallest saplings, one of the second four tallest, etc. The groups were arranged randomly in the greenhouse.

Each sapling in two of the groups was dosed once only on 30th July 2002 with 200 ml of 1000 μg l⁻¹ TCA solution (i.e. a total of 200 μg TCA) applied either to the soil surface (group TCA-S), or sprayed as a fine mist to the foliage (group TCA-F). The application volume was chosen to correspond to ~2 mm precipitation depth over the projected canopy surface area (the estimated canopy retention depth before throughfall). The dose rate was therefore equivalent to ~2000 μg TCA m⁻². Although this TCA concentration is not typical of those found in natural precipitation, it is comparable to the annual ambient wet deposition flux of ~1000 μg TCA m⁻² measured at an upland Sitka spruce site in Scotland (Heal et al., 2003).

Two control groups of saplings (CON-S and CON-F) were dosed in the same manner with deionised water.

To avoid contamination by spray drift, the TCA-F saplings were removed to another greenhouse for spraying and returned when dry. All plants, including those in soil-dosed groups, had a paper plate with water repellent coating placed around the base of the stem to prevent drips from the spray applications from contaminating the soil, and to ensure parity in soil moisture retention for all saplings. The possibility that some of the spray solution applied to the foliage may have found its way into the soil via stem flow cannot be absolutely excluded but any contribution will have been small. In addition, it is inevitable that a proportion of spray solution will not have landed on the foliage but have been lost by drift.

Plants were irrigated with water twice a week on average via plastic saucers beneath their pots. This water was shown to contain low levels of background TCA.

Samples of the current year needles (year class C) were collected from all plants immediately prior to treatment (t = 0 days), then approximately twice a week for 6 weeks and weekly until t = 85 days on 23 October 2002 (a total of 17 sampling occasions). Needles were always collected from branches on the second whorl of each sapling, and pooled within each group, i.e., for every sampling occasion, the needle material always included needles from each sapling in that group. Sampling is obviously destructive so it was not possible to resample the same needles at each time point. It was also necessary to pool needle material to avoid excessive physical damage to the saplings in an experiment requiring removal of foliage on 17 occasions. Therefore reported TCA values reflect both analytical variability and within-group sapling variability. Material was deliberately sampled from the same whorl on each occasion in order to control for one possible source of sampling variability. This precaution has not been reported in previous studies.

Samples of the previous year’s needles (C+1) were similarly collected on days 34 and 85.

Prior to analysis, shoots were immersed in deionised water, ultra-sonicated for 5 min, rinsed, and the excess water removed by tissue to ensure that measured TCA was internal to the needle matrix and not adsorbed to the surface. Needles were stripped from the branch and homogenised by grinding frozen under liquid nitrogen with a pestle and mortar, to completely release TCA from the needle matrix.

TCA was quantified by the method of thermal decarboxylation to chloroform developed by Plumacher and Renner (1993). The homogenised needles (1 g) were heated to 100°C in sealed 20 ml headspace vials for 90 min to convert TCA to chloroform. The vials were re-equilibrated at 60°C and the chloroform quantified against aqueous TCA standard solutions, undergoing the same process, using headspace sampling and gas chromatography with ECD detection. Parallel samples equilibrated at 60°C only provided the background chloroform present in the needles. Further details are provided in Heal et al. (2003) and Cape et al. (2003). It was necessary to correct the measured TCA concentration in needle samples for the different degree of partitioning of chloroform between headspace and needle matrix or water matrix. The ratio of these partition factors was derived from standard addition experiments in which a series of concentrations of TCA solution were added either to water or to needle. Example results are shown in Fig. 1. The ratio of the
3. Results and discussion

The concentration of TCA in the needles of the control group saplings, CON-S and CON-F, are shown in Fig. 2. The variation in concentrations provide an estimate of the within-group experimental and analytical variability in measurement of TCA needle concentration in the absence of TCA dosing. Fig. 2 shows there was no significant trend with time in needle TCA concentrations for either CON group, although mean CON-S and CON-F concentrations did differ significantly. The presence of TCA in the needles of the CON saplings is the result of previous background exposure over the entire lifetime of all saplings from irrigation tapwater shown to contain TCA (Cape et al., 2003). The variability in the CON-F and CON-S needle concentrations with time (and presumably similar variability in the TCA treated saplings) reflects intrinsic variability in TCA content of shoots both within-sapling and between-sapling. Within- and between-tree variability of TCA concentration in environmental foliage is well-documented. The origin of the mean difference between CON-S and CON-F concentrations presumably reflects chance between-group differences when the 40 saplings were divided into groups of 10 at the start of the experiment.

The TCA concentrations in the needles of the TCA-S and TCA-F groups following the single application of TCA at \( t = 0 \) to soil or foliage only are shown in Figs. 3a and b, respectively. These values are analysed with respect to the change from \( t = 0 \) using the variation in the CON sapling values as the estimate of the likely variation in the treated saplings. As indicated above, this latter variation includes both experimental (i.e. sapling/sampling) variability as well as analytical variability. The mean ± s.d. concentration of TCA in the year C needles of the CON-S and CON-F saplings were \( 22 ± 6 \, \text{ng g}^{-1} \text{fwt} \) (\( n = 9 \)) and \( 14 ± 5 \, \text{ng g}^{-1} \text{fwt} \) (\( n = 9 \)).

gradients in Fig. 1, termed the partition ratio, is a constant for a given set of headspace conditions (volume of vial, mass of matrix, headspace aliquot analysed, etc.), regardless of any run-to-run variability in ECD response sensitivity. Standard addition plots as shown in Fig. 1 were repeated on a number of occasions yielding a mean partition ratio of 1.94 (s.d. = 0.26, \( n = 8 \)). This partition ratio was used to correct needle sample TCA values quantified on subsequent GC runs against just a water calibration plot.

The decarboxylation method has the advantage of being a whole-sample technique whereas extraction-derivatisation methods must assume that all intrinsic matrix-bound TCA is extracted into solution. However, the decarboxylation method does not directly quantify TCA, although samples are always blank-corrected for chloroform quantified at 60°C so that only chloroform produced by the sample after 1.5 h at 100°C is quantified as TCA. The linearity of the two plots in Fig. 1 demonstrate the applicability of the standard addition methodology, including the correction for any background chloroform prior to decarboxylation.

All samples were analysed in triplicate. All TCA concentrations were expressed per fresh wt needle. The water content of the needles (mean 58.1 ± 1.8% s.d., \( n = 25 \)) did not vary significantly with sampling date or treatment type, so this parameter did not introduce any bias to the analyses.

\[ n = \frac{3}{4} \]

\[ t = \frac{0}{0} \]

\[ r = \frac{0}{0} \]

\[ y = 38780x + 8131 \]

\[ y = 19360x + 288240 \]

\[ y = 38780x + 8131 \quad r = 0.998 \]

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For TCA-S data, curves are model fits to the data of the kinetic scheme shown as determined using the control group data in Fig. 2. The solid horizontal lines in each figure are taken from each of the 10 saplings within a group on each sampling occasion. The error bars are standard deviations of analytical triplicates of pooled needle material from each of the 10 saplings within a group on each sampling occasion. The horizontal lines in each figure are the deviation of analytical triplicates of pooled needle material foliage only (group TCA-F, (b)). The error bars are standard deviation of analytical triplicates of a pooled sample from all 10 saplings in the group. The additional variability with time reflects the sampling variability discussed above for the CON groups.

It is not possible to compare directly the net accumulation of TCA in needles of the TCA-S and TCA-F treatments because the exact proportion of TCA solution intercepted by foliage in the spray-treated batch is not known. However, the existence of independent below-ground and above-ground pathways of TCA uptake is unequivocal, as is observation of uptake from a few days after application.

The observed time-dependence in needle concentration in Fig. 3 is typical of a sequential kinetic system involving TCA uptake into the needles from an initial “reservoir” (the TCA dose) and elimination from the needles, presumably by metabolism/detoxification. In addition, the existence of a competing parallel loss from the initial reservoir due to loss of TCA in the soil, or on the foliage surface, before uptake into the sapling, must also be included. In this context, loss means not available for uptake into the needles. This could include chemical and/or biological degradation and/or immobilisation and, for the soil application route, the possibility of permanent leaching from the soil into the irrigation saucer (although this cannot be the case for the foliage application route).

The data were modelled according to the simple kinetic scheme illustrated in Fig. 4, in which mass transfer from each compartment was assumed to follow first-order kinetics, and the first-order rate constants were the fitting parameters. Example fits to needle TCA concentration with time are shown in Fig. 3; fitted rate constants are quoted in the caption. The inverse of each rate constant is the lifetime for the associated transfer process. A nominal fresh mass of 130 g needles respectively. The 95% confidence interval for measurement of needle TCA concentration at a single time point is 1.96 times the s.d. concentration. This yields 95% confidence interval factors for within-group variation, relative to the corresponding mean, of 0.4–1.6 and 0.3–1.7 for soil and foliage treatments, respectively. These 95% confidence values about the t = 0 values, are also shown in Figs. 3a and b.

Figs. 3a and b clearly show there was significant uptake and subsequent elimination of TCA in needles of both soil-dosed (TCA-S) and foliage-dosed (TCA-F) saplings. In particular, concentrations in needles of the TCA-S group remain significantly higher than t = 0 even after 85 days. For the TCA-S saplings, needle TCA concentrations increased from 18 ± 3 ng g⁻¹ fwt at t = 0, to a maximum of 60 ± 2 ng g⁻¹ fwt after 31 days, decreasing to 33 ± 2 ng g⁻¹ fwt after 85 days. The change in TCA in needles of TCA-F saplings was lower, but still significant, increasing from 14 ± 3 ng g⁻¹ fwt⁻¹ at t = 0, to a maximum of 40 ± 2 ng g⁻¹ fwt⁻¹ after 28 days, then decreasing to 15 ± 1 ng g⁻¹ fwt⁻¹ after 85 days. (The errors quoted are 1 s.d. of analytical triplicates of a pooled sample from all 10 saplings in the group.) The 95% confidence values about the t = 0 values, are also shown in Figs. 2.
for a six-year Sitka spruce sapling was derived from the data of Cannell et al. (1983), and validated by destruction of one sapling at the end of the experiment.

Not all TCA dosed to the soil or the foliage is likely to be “available” for uptake, so the initial dose is not actually a well-characterised fixed parameter. (Some of the foliage dose will have been lost by drift or canopy drip, whilst some of the soil dose may have leached out of the pot.) In practice, the kinetic fitting was fairly insensitive to assumed values of initial available TCA between 150 and 200 µg per plant although, overall, the model was not very tightly constrained. Despite this, the following conclusions emerge from model fits. First, the data are best fitted when there is provision for substantial net loss of initial TCA in parallel to uptake into the needles. In the case of the soil-dosed experiment, this is taken to indicate chemical/biological degradation of TCA in soil and is consistent with evidence from chronic sapling exposure studies (Cape et al., 2003; Dickey et al., 2003) and unpublished lysimeter measurements from this group, which all show that TCA added to soil is rapidly degraded on a timescale of a few days to weeks. For the foliage-dosed experiment, this loss is presumed to be similar degradation on the foliage surface.

Secondly, the values obtained from the fits, both for the kinetic parameters and the proportions of TCA passing through each compartment, are broadly consistent between both application regimes. Uptake of TCA into the needles has a first-order lifetime of several 100 days (± ~100 days). This parameter is not of relevance to environmental situations since trees are continuously exposed to “fresh” TCA directly to the canopy or from the soil via TCA in precipitation and, possibly, soil sources. The model estimates of first-order lifetimes corresponding to within-forest TCA concentration rate, and the loss rate from the initial reservoir in parallel to uptake, are ~70 ± 40 days and ~20 ± 10 days, respectively. (Quoted ranges are approximate 95% confidence intervals obtained in the non-linear fitting model and are large because of difficulties in constraining the model.)

Thirdly, very approximately, after the 85 days of observation, the kinetic fits suggest ~1% of initial TCA dose applied per sapling was present in the needle foliage, ~2–7% remained in the soil or externally on the foliage, ~89–95% was lost externally before uptake, and ~2–3% was eliminated within the needles. Regardless of kinetic modelling, it is evident from Fig. 3 that only a small proportion (<1%) of the initial dose of TCA applied is present in the needles after 85 days.

The needle TCA elimination half-life of ~50 days applies to an actively growing plant. In a separate study, elevated TCA concentrations in Sitka spruce seedlings at the end of the growing season in October were reduced by only about one-third by April the following year (Dickey et al., 2003) indicating very little detoxification metabolic activity over winter, as expected. The elimination rate reported here is slower than that observed in Scots pine seedlings by Sutinen et al. (1997) who noted that during the four weeks after repeated exposure to TCA, needle concentrations decreased from a peak of 250 ng g⁻¹ to 36 ng g⁻¹ fwt.

The analysis of older needles (year class C + 1) sampled after 34 and 85 days clearly showed the presence of TCA in older needles via both application routes. On both sampling occasions, and for both treatments, concentrations in C and C + 1 needles were not significantly different (taking into account the natural sampling/analytical variability discussed for Figs. 2 and 3), although there was a (non-significant) trend for concentrations greater in C + 1 needles than in C needles for foliage application route. (N.B. n = 2 data only.)

The observation of a direct canopy route of uptake of TCA from solution initially appears surprising given the highly hydrophobic nature of the needle cuticle. The measurements are not simply residual TCA on the needle surface because needles were thoroughly rinsed in water before analysis. Further field evidence of canopy uptake from measurements in a mature Sitka spruce forest has been shown by this group (unpublished). A laboratory study has shown no direct partitioning of TCA from solution through the needle cuticle (Cape et al., 2003), as is also the case for other anions, e.g. SO₄²⁻ (Percy and Baker, 1989). However, elevated TCA concentrations have been observed in the branchwood of foliage-treated saplings indicating that transfer through the branchwood is the likely uptake route (Cape et al., 2003). Recently, Benesch and Gustin (2002) have also demonstrated an above ground route for accumulation of trifluoroacetic acid (TFA) in needles of Pinus ponderosa saplings following repeated exposure to mists of TFA solution.

An observational assessment of external sapling health (categorisation according to foliage density and colour) revealed no observable short-term adverse health effects arising from application of this single dose of TCA. However, absence of visual damage does not imply absence of toxicity; physiological and biochemical changes, if causal, are likely to result from chronic exposure to environmental levels of TCA (Cape et al., 2003; Dickey et al., 2003).

In conclusion, this work has demonstrated uptake of TCA into Sitka spruce saplings via above-ground only and below-ground only routes. The former is important given the considerable proportion of wet precipitation intercepted by the forest canopy. The retention of TCA in foliage is relatively long-lived after exposure by either route, with a half-life of approximately 7 weeks during the growing season.
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