EFFECT OF TEMPERATURE ON THE UPTAKE AND METABOLISM OF CYANIDE BY WEEPING WILLOWS

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Plants’ uptake and metabolism of cyanide in response to changes in temperature was investigated. Pre-rooted weeping willows (Salix babylonica L.) were exposed to hydroponic solution spiked with potassium cyanide for 2–3 d. Ten different temperatures were used, ranging from 11°C to 32°C. Cyanide in water, plant tissue, and air was analyzed spectrophotometrically. The results revealed that significant amounts of the applied cyanide were removed from the aqueous solutions in the presence of plants. Small amounts of free cyanide were detected in plant materials in all treatments, but there was no clear trend that showed an increase or decrease in the accumulation in plant material with temperature. The highest cyanide metabolism rate for weeping willows was found at 32°C with a value of 2.78 mg CN/(kg·d), whereas the lowest value was 1.20 mg CN/(kg·d) at 11°C. The temperature coefficient, Q₁₀, which is the ratio of metabolism rates at a 10°C difference, was determined for weeping willows to be 1.46. In conclusion, changes in temperature have a substantial influence on the uptake and metabolism of cyanide by plants, but cyanide accumulation does not increase with temperature.

KEY WORDS: cyanide, metabolism, phytoremediation, temperature, weeping willows

INTRODUCTION

Free cyanide (HCN or CN⁻) is one of the most rapidly killing toxicants (Faust, 1994). Despite this, it is a high-volume production chemical with a wide variety of uses. The annual production of cyanide hydrogen (HCN) is 1.4 million metric tons and cyanide compounds are used in the production of adhesives, computer electronics, fire retardants,
cosmetics, dyes, nylon, paints, pharmaceuticals, plexiglas, rocket propellant, road and table salts, and marzipan (Mudder and Botz, 2001). Approximately 13% of the produced cyanide is used in gold mining for heap leaching, which means an environmentally open use. A couple of disastrous events with cyanide compounds, such as the accidental release of methyl isocyanate in the Union Carbide manufactory in Bhopal, India, in December 1984, (Sriramachari and Chandra, 1997), the cyanide spill at Baia Mare, Romania, in 2000 (Korte, Spiteller, and Coulston, 2000, UNEP/OCHA, 2000), repeated cyanide spills from the Ashanti gold fields in Ghana, and accidents in China (Anane, 2001; Li and Zhen, 2000), to mention some, have raised great interest in the environmental fate and treatment strategies for cyanide compounds.

There are several metabolic pathways for free cyanide (Ebbs, 2003). Vascular plants possess an enzyme that can metabolize free cyanide by the enzyme beta-cyanoalanine synthase, which connects HCN to L-cysteine and produces beta-cyanoalanine (Blumenthal et al., 1968). The final metabolite is asparagine, an important amino acid. The activity of the enzyme beta-cyanoalanine synthase is several orders of magnitude higher than the cyanide produced by the ethylene synthesis (Manning, 1988).

The common feature of all vascular plants to metabolize free cyanide was used for a series of studies to uncover the kinetics of the plant-detoxification system and the relation to uptake and toxicity. Ebbs et al. (2003) used free and iron-complexed cyanide labeled with $^{15}$N. The authors found that free cyanide was rapidly taken up by plants and subsequently metabolized. There also was evidence for an uptake of ferrocyanide, probably followed by metabolism inside plants. Larsen, Trapp, and Pirandello (2004) tested the toxicity and metabolism rate of free cyanide in five woody plants. The kinetics showed signs of enzyme limitation and followed Michaelis–Menten kinetics. The metabolism rate of basket willow was the highest of the tested species, with about 10 mg CN/(kg fresh weight·h) for roots. Furthermore, willows were able to take up ferro- and ferricyanide and seemed to metabolize the complex (Larsen and Trapp, 2006).

Studies on the toxicity and metabolism of cyanide were also performed with Chinese plants. These studies confirmed the cited findings on toxicity (Yu, Trapp, and Zhou, 2005 a), but gave also new insights.

Twenty-eight-Chinese plant species from 23 families were tested for their capacity to remove cyanide in a closed-bottle test (Yu et al., 2004). All species were found to metabolize cyanide, but with different velocities. Data on the Michaelis–Menten kinetics (half-saturation constant $K_M$ and maximal removal rate $v_{max}$) of cyanide removal for 12 plant species out of 9 families was determined by using excised leaf tissues (Yu et al., 2005b). Values of $v_{max}$ and $K_M$ were found in a range between 6.68 and 21.91 mg CN/(kg·h) and 0.90 to 3.15 mg CN/L, respectively. The values of all $v_{max}$ were normally distributed with a mean of 13 mg CN/(kg·h).

Bushey, Ebbs, and Dzombak (2006 a) and Bushey et al. (2006b) developed a model for the calculation of the uptake and metabolism of free and iron-complexed cyanide and fitted a parameter set to the experimental results of Ebbs et al. (2003) and additional data. Larsen, Ucisik, and Trapp (2005) developed a mathematical model for the fate of cyanide in plants, considering uptake and the Michaelis–Menten removal kinetics. The model predicts a non-linear relationship between the external concentration of cyanide and the accumulation in plants: below a certain level, plant enzymes can remove the cyanide. No accumulation and no toxic effects occur. Above a certain threshold (approximately 20 mg CN/L for willows grown in sand), the enzyme system of the plant is overloaded and the accumulation of cyanide is followed by toxic effects. This means that the metabolism rate
of plants plays a key role in accumulation and toxicity. Removal rates were measured for
detached plant materials in the closed-bottle test, but could also be determined by fitting
the model (inverse modeling). It turned out that the results for metabolism from detached,
but otherwise intact, plant materials gave valid model predictions in this case.

All studies cited above were done at room temperature (between 20 and 25°C).
However, temperatures in the environment vary. For most plants, the fastest growth occurs
between 15 and 30°C (Larcher, 1995). Temperature affects the transpiration, growth,
and metabolism of plants and, therefore, both the uptake and elimination of pollutants.
For phytoremediation projects, low temperatures mean slow growth and slow uptake of
chemicals and can, therefore, prolong the time period required to reach cleanup goals. High
temperatures increase the transpiration of plants. Contaminants are translocated faster. This
may result in higher accumulation of toxicants, eventually followed by phytotoxic effects
or an increased risk of contamination for consumers of plant materials. However, parallel
to the increase in transpiration, metabolism rates may also increase. Therefore, the eventual
accumulation of toxic residues depends critically on changes in transpiration and metabolic
rates with temperature.

The influence of temperature on the cyanide metabolism rate of detached leaves was
determined in closed bottles (Yu et al., 2005c). For Chinese elder, the highest cyanide
removal rate was found at 30°C with a value of 12.6 mg CN/(kg·h), which was over
3-fold higher than at 11°C. The cyanide removal rate of weeping willow at 32°C (9.72 mg
CN/[kg·h]) was almost five times higher than at 11°C (1.97 mg CN/[kg·h]). This means
that the effect of temperature on cyanide metabolism was more important than the choice
of plant species. In this study, the influence of temperature on the uptake, accumulation,
and metabolism of cyanide by intact weeping willows was investigated. The reasons for
the influence of temperature on the metabolism rate are discussed.

MATERIALS AND METHODS

Trees, Transpiration, and Exposure Regimes

Weeping willow cuttings (Salix babylonica L.) were sampled from nature on the
campus of the Hunan Agricultural University, Hunan, China. Tree cuttings (40-cm length)
were removed from a mature single specimen and placed in buckets of tap water at room
temperature (15–20°C) under natural sunlight until new roots and leaves appeared. After
a 2-mo growth period, pre-rooted cuttings were transferred to a 250-mL Erlenmeyer flask
filled with approximately 250 mL of aerated aqueous cyanide solution (deionized oxygen-
saturated water). The concentration of spiked solutions was 0.98 mg CN/L (± 0.038 mg
CN/L). The flasks were all sealed with cork stoppers and play dough to prevent the escape
of water or chemicals, and then wrapped with aluminum foil to inhibit algae growth. The
flasks were put in a climate chamber with constant temperatures of 11, 14, 17, 20, 22,
24, 26, 28, 30 and 32°C and a relative humidity of 60 ± 2% under continuous artificial
light. Modified ISO 8692 nutrient solution was used to support tree growth. The pH of the
cyanide solution was adjusted to 6.9 with sodium hydroxide. Six separate flasks containing
willows and aqueous cyanide were conducted for each treatment temperature. One control
in four replicates contained plant cuttings, but no cyanide, to compare transpiration to
treated trees, and to determine background cyanide concentrations. A second control in two
replicates (the flasks were also wrapped with aluminum foil and sealed with cork stoppers)
contained cyanide, but no plant cuttings, to quantify the effects of loss during handling or
by volatilization, hydrolysis, and/or degradation by microorganism. The concentration of cyanide in the aqueous solution was measured before the tree cuttings were transferred. Exposure periods were 3 d for the 11, 14, 17, 20 and 22°C treatments and 2 d for the 24, 26, 28, 30 and 32°C treatments, respectively. At the end of the test, the remaining solution, roots, leaves, and top and bottom stem were analyzed for cyanide.

The toxic effect of cyanide solutions was evaluated by measuring transpiration in trees. The transpiration of plants is coupled to photosynthesis and an inhibition of transpiration is a reliable and rapid hint of toxic effects (Trapp et al., 2000). The transpiration of water was determined from the weight loss of the system (flask with solution and plant) during the experiment and expressed as absolute transpiration (g/d).

Chemical Analysis

A stock solution with a concentration of 0.92 g CN/L was prepared using potassium cyanide. The concentration of cyanide in the aqueous solution was determined photometrically by a standard method (State Environmental Protection Administration of China, 1989; method GB 7487–87). A 1–10-mL solution of the samples (depending on the expected concentration of cyanide) were pipetted into a 25-mL colorimetric cylinder and 1% sodium hydroxide was added to the mark of 10 mL. Then, 5.0 mL of buffer solution with potassium dihydrogen phosphate and sodium phosphate were added. Quickly, 0.2 mL of chloramine-T solution with the concentration of 1% (volume/mass) was added. The vessel was closed with a stopper and left standing for 3–5 min. Five milliliters of the color reagent (a mixture of isonicotinic acid and 3-methyl-1-phenyl-5-pyrazolone) were added. The sample was diluted with water to 25 mL and mixed. During the analysis, the colorimetric cylinders were all kept in a water bath with a temperature of 32°C for 40 min. The absorption of light at 638 nm was measured in a cell of optical path length of 10 mm against a water reference.

The standard solution of 0.92 mg CN/L (0.10, 0.20, 0.50, 1.0, 2.0, and 5.0 mL) was pipetted into seven different colorimetric cylinders (25 mL) and 1% sodium hydroxide was added to the mark of 10 mL. The remaining procedure was identical to that described earlier. The data obtained were used to derive the standard curve.

All chemicals used were per analysis (p. a.) grade (in China, p. a. grade is >99.5% purity), except potassium cyanide and nicotinic acid, which were technical grade (92–95% purity). The stock and standard solutions of KCN used in this test were calibrated by a standard solution of AgNO₃, which is also calibrated by a standard solution of NaCl (State Environmental Protection Administration of China, 1989). The detection limit of this method was determined from blank + 3 standard deviations of 10 replicates to be 0.004 mg CN/L with a 10-mL sample volume.

The analysis of cyanide in plant tissues was also performed by a standard distillation method (State Environmental Protection Administration of China, 1989; method GB 7486–87). Five milliliters of 1% sodium hydroxide was poured into the absorption vessel of the distillation unit. Fresh plant materials (2.0–10 g, depending on the harvested weight of plant tissues) was cut into pieces and placed into a 500 mL round bottom flask, and 100 mL of distilled water was added. Then, 1 ml of zinc acetate with the concentration of 10% (volume/mass) and 10 mL of tartaric acid (15% volume/mass) were added before heating and mixing. Approximately 50 mL of the distilled solution containing cyanide from plant materials were collected, quantitatively transferred to a 50-mL volumetric flask, and made up to the volume with water. The solution was stored below 4°C until the concentration of the cyanide was determined as described. All samples were analyzed with a maximum hold time of 4 h.
Cyanide Transpired by Plants

Transpired cyanide was measured using a refined test chamber. Treated plants were prepared as described above with the entire 250-mL Erlenmeyer flask enclosed in a glass chamber (20 × 20 × 50 cm) with air flowing through at 24°C. The tube at the outflow of the vessel was connected to a gas trap containing 5 mL sodium hydroxide of 1% to trap airborne cyanide. The gas trap tube was wrapped with aluminum foil and changed daily, after which all gas tubes were analyzed for cyanide. The duration of this test was 2 d.

Determination of the Removal Rate

In the absence of volatilization, negligible background cyanide in controls with plants and, if the loss from controls without plants is negligible, all loss from the system can be attributed to removal by plants. The removal velocity \( v \) (mg CN/(kg·d)) was calculated from final and initial mass using the formula

\[
 v = \frac{m(t) - m(0)}{\Delta t \times M}
\]

where \( m(t) \) is the mass (mg) of cyanide in solution plus plant (sum of leaves, roots, and stem) at time \( t \) and \( m(0) \) is the mass of cyanide in the solution at the beginning; \( \Delta t \) is the time period (d) and \( M \) is the mass of the plant (kg).

Determination of the Temperature Coefficient \( Q_{10} \)

The influence of temperature on the removal rate was quantified by calculating the temperature coefficient \( Q_{10} \), which is defined as the increase of the metabolic activity over a 10°C increase in temperature. The temperature coefficient was derived using the equation from Atkin, Zhang, and Wiskich (2002):

\[
 Q_{10} = 10^{\Delta T \times \text{slope}}
\]

where \( \Delta T \) is 10°C and \( \text{slope} \) is the slope of the linear fit curve of log \( v_P \) versus temperature \( T \).

Statistical Methods

The students \( t \)-test (two-tailed) and the Pearson’s product–moment correlation and regression were done in Excel. The significance of the correlations was judged using tabled values for critical \( r \) (the degree of freedom is \( n-2 \) and the significance level \( \alpha \) was 0.01 or 0.05) from Sachs (1992). The partial correlation was used to determine whether a correlation between two variables was due to a common correlation to a third variable by using

\[
 r_{xy,z} = \frac{r_{xy} - r_{xz} \times r_{yz}}{\sqrt{(1 - r_{xz}^2) \times (1 - r_{yz}^2)}}
\]

where \( r_{xy,z} \) is the partial correlation coefficient between variables \( x \) and \( y \) under the assumption of a constant variable \( z \) and \( r_{xy} \) is the bivariate Pearson correlation coefficient between variables \( x \) and \( y \), etc.
RESULTS

Removal of Cyanide from Hydroponic Solution by Willows

Figure 1 gives the changes of concentrations of cyanide in the hydroponic solutions with weeping willows at different treatment temperatures. In the control with cyanide in the absence of plants, negligible changes of cyanide in the solution (data not shown) were found at all treatment temperature groups over the entire exposure periods. This indicated that the disappearance of cyanide from solutions in flasks with plants could be attributed to the uptake by willows. In all treatments, significant amounts of cyanide in hydroponic solution were removed in the presence of weeping willows. The cyanide in solution fell from 0.98 (±0.045) to 0.43 (±0.042) mg CN/L at 11°C during the 3-d exposure. At the highest treatment temperature (32°C), cyanide was reduced from 0.99 (±0.032) to 0.13 (±0.098) mg CN/L during 2-d exposure. Only between 8.7 and 36.3% of cyanide could be recovered from solution and plant material.

Uptake of Cyanide into Plants

Figures 2a–2d show the concentrations of cyanide in plant tissues at different treatment temperatures. Cyanide was detected in plant materials in all treatment groups, confirming the uptake of cyanide into plants. The cyanide background in unexposed control trees was 0.023 mg CN/kg for roots, 0.038 mg CN/kg for leaves, and 0.015 mg CN/kg for stem (n = 2 for all controls). The concentrations in the respective control solutions were below the detection limit of 0.004 mg CN/L. Compared to controls, only the cyanide levels in roots and bottom stem of exposed plants were elevated. The concentration ratios between plant and initial solution can directly be seen from Figures 2a-2d (because the concentration in initial solution was 0.98 mg/kg, practically 1). The concentration ratio between root and initial solution (root concentration factor [RCF]) is falling with the temperature from 11 to 22°C (3-d exposure) and again from 26 to 32°C (2-d exposure). The linear trend is significant at $\alpha = 0.05$. For top and bottom stems (stem concentration factor [SCF]) as well as for leaves (leaf concentration factor [LCF]), there is no clear and significant trend. Related to final concentrations in solution, the RCF decreases with temperature, while SCF and LCF increase, but none of the trends is significant ($\alpha = 5\%$).
Figure 2 Measured cyanide concentrations (mg CN/kg fresh weight) in a) roots, b) leaf, c) top stem, and d) bottom stem of weeping willows (*Salix babylonica* L.) at different treatment temperatures. The exposure period was 3 d for the treatments from 11°C to 22°C and 2 d for the treatments from 24°C to 32°C. The values are the mean of six replicates. Error bars represent the standard deviation; FW = fresh weight.
Mass Balance of Cyanide

After uptake from solution and translocation to the upper stem and leaves, cyanide may have been volatilized. However, no cyanide above the detection limit was trapped for willows exposed to 24°C over a 2-d test period using the test chamber with the gas trap (cyanide transpired at other treatment temperatures was not measured). The same result was also found in the study by Ebbs et al. (2003). Trapp, Larsen, and Christiansen (2001) found small amounts (1.5%) of cyanide in the air. Therefore, cyanide loss into air was neglected in the mass balance given in Table 1 and only cyanide in plant tissues and in the solution was considered. Among plant materials, the largest amount of cyanide was found in the roots, followed by the bottom stem. The lowest cyanide concentration was found in leaves, where all values were very close to the background in controls (0.038 mg/kg). The cyanide recovered from plant biomass accounted for maximally 2% of the initially applied mass. Only between 8.7 and 36.3% of cyanide could be recovered from the final solution and plants. Loss from controls without plants was negligible; therefore, it is most likely that all loss was due to metabolism by plant enzymes. This is consistent with earlier findings (Ebbs et al., 2003; Larsen et al., 2004, 2005; Yu et al., 2004). The calculated cyanide metabolism rates are shown in Table 1; the rates increased with temperature. The highest cyanide metabolism rate was found at 32°C with a value of 2.78 mg CN/(kg·d), which was more than 2-fold higher than at 11°C.

Response of Plant Transpiration to Cyanide Exposure at Different Temperatures

Figure 3 shows the absolute transpiration of control trees (without exposure to cyanide) as compared to exposed trees in the temperature range from 11°C to 32°C. A slight difference in transpiration between treated and non-treated plants was found, probably stemming from differences in individual trees. The difference is generally small and not significant (two-tailed t-test, significance level α = 0.05). In some cases, exposed trees show the higher transpiration. No attempt was made to select homogeneous plant materials; therefore, the transpiration is subject to variations. The results also indicate that, with the increase in temperature, the transpiration of plants generally increased, as expected. There also were no significant differences in the plant growth between treated and non-treated plants (data not shown). Symptoms of chlorosis of leaves were not observed in

![Figure 3](image_url)
Table 1 Cyanide mass balance. Values are the mean of six replicates (in parenthesis: standard deviation). The exposure period was 3 d for the treatments from 11°C to 22°C and 2 d for the treatments from 24°C to 32°C

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Initial</th>
<th>Final</th>
<th>Roots</th>
<th>Leaves</th>
<th>Top stem</th>
<th>Bottom stem</th>
<th>Cyanide Loss (%)</th>
<th>Metabolism rate (mg CN/kg·d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>245.6 (11.20)</td>
<td>84.0 (9.97)</td>
<td>2.30 (0.674)</td>
<td>0.35 (0.09)</td>
<td>0.53 (0.12)</td>
<td>1.98 (0.60)</td>
<td>63.7</td>
<td>1.20 (0.18)</td>
</tr>
<tr>
<td>14</td>
<td>248.0 (6.69)</td>
<td>56.95 (14.81)</td>
<td>1.87 (0.51)</td>
<td>0.17 (0.01)</td>
<td>0.40 (0.19)</td>
<td>0.89 (0.27)</td>
<td>75.7</td>
<td>1.43 (0.36)</td>
</tr>
<tr>
<td>17</td>
<td>264.5 (11.43)</td>
<td>37.4 (18.89)</td>
<td>1.12 (0.73)</td>
<td>0.2 (0.12)</td>
<td>0.56 (0.17)</td>
<td>1.09 (0.29)</td>
<td>84.7</td>
<td>1.55 (0.49)</td>
</tr>
<tr>
<td>20</td>
<td>248.0 (6.69)</td>
<td>56.95 (14.8)</td>
<td>1.36 (1.04)</td>
<td>0.03 (0.02)</td>
<td>0.40 (0.18)</td>
<td>0.79 (0.30)</td>
<td>77.0</td>
<td>1.87 (0.62)</td>
</tr>
<tr>
<td>22</td>
<td>243.7 (13.33)</td>
<td>18.37 (14.0)</td>
<td>1.32 (0.41)</td>
<td>0.07 (0.07)</td>
<td>0.15 (0.04)</td>
<td>1.37 (0.46)</td>
<td>91.3</td>
<td>1.97 (0.52)</td>
</tr>
<tr>
<td>24</td>
<td>246.9 (2.80)</td>
<td>23.1 (14.8)</td>
<td>1.02 (0.38)</td>
<td>0.3 (0.13)</td>
<td>0.63 (0.20)</td>
<td>0.53 (0.28)</td>
<td>89.6</td>
<td>2.02 (0.28)</td>
</tr>
<tr>
<td>26</td>
<td>236.6 (3.65)</td>
<td>56.2 (8.65)</td>
<td>1.41 (0.19)</td>
<td>0.06 (0.02)</td>
<td>0.15 (0.03)</td>
<td>1.69 (0.60)</td>
<td>74.9</td>
<td>2.15 (0.35)</td>
</tr>
<tr>
<td>28</td>
<td>236.6 (3.65)</td>
<td>56.2 (8.65)</td>
<td>1.19 (0.53)</td>
<td>0.06 (0.02)</td>
<td>0.22 (0.06)</td>
<td>0.69 (0.07)</td>
<td>78.4</td>
<td>2.27 (0.38)</td>
</tr>
<tr>
<td>30</td>
<td>239.5 (6.16)</td>
<td>45.2 (19.12)</td>
<td>0.92 (0.11)</td>
<td>0.21 (0.07)</td>
<td>0.59 (0.24)</td>
<td>1.37 (0.36)</td>
<td>79.8</td>
<td>2.62 (0.96)</td>
</tr>
<tr>
<td>32</td>
<td>246.9 (8.06)</td>
<td>23.7 (18.1)</td>
<td>0.82 (0.26)</td>
<td>0.35 (0.15)</td>
<td>0.33 (0.14)</td>
<td>0.86 (0.37)</td>
<td>89.4</td>
<td>2.78 (0.95)</td>
</tr>
</tbody>
</table>
any plant, giving rise to the conclusion that the cuttings maintained normal physiological functioning over the entire test period without any measurable and observed toxic effects.

**Temperature Coefficient $Q_{10}$**

The response of enzymatic reactions to a temperature increase can be summarized by two functions: an increase of the forward reaction and a decay due to enzyme denaturation as the temperature rises (Raison, 1980). Figure 4 shows the fit line ($r^2 = 0.983$) from which a temperature coefficient $Q_{10}$ of 1.46 was calculated. This value is in the range of previously reported $Q_{10}$ values of 1.1 to 2.9 (Atkin *et al.*, 2000; Azcón-Bieto, 1992) for metabolic processes.

**DISCUSSION**

The removal rates from this study can be compared to rates found earlier, but which were derived from removal tests in closed bottles. For weeping willow *Salix babylonica* L., Yu *et al.* (2004) found a removal capacity of 6.08 mg CN/(kg·h) with an initial concentration in solution of 0.94 mg CN/L in a test with detached leaves in a closed bottle. The same test was performed with varying concentrations in solution (0.49, 1, 2.07, and 7.59 mg CN/L) to determine the Michaelis–Menten parameters (Yu *et al.*, 2005b). For *Salix babylonica* L., the removal rate at 1.0 mg CN/L was 4.1 mg CN/(kg-h), while $v_{\text{max}}$ was 9.57 to 12.76 mg CN/(kg-h). For the closely related species basket willow (*Salix viminalis*), Larsen *et al.* (2004) found $v_{\text{max}}$ for leaves from 9.3 to 9.4 mg CN/(kg-h) and for roots from 10.7 to 14.5 mg CN/(kg-h). In later experiments, Larsen *et al.* (2005) found a $v_{\text{max}}$ for basket willow leaves of 9.6 mg CN/(kg-h) and for roots of 6.9 mg CN/(kg-h).

To compare these earlier results from closed-bottle tests with the results obtained here with intact trees, it has to be considered that the plant mass in contact with solution is different. Typical willow cuttings have approximately 1–2-g roots, a 35–40-g stem, and 1–2-g leaves. Therefore, the ratio of root to total plant mass is approximately 1:20 to 1:40 and a 20 to 40 times faster removal rate can be expected with detached plant material in the closed-bottle test.

For 22°C, a rate of 1.97 mg CN/(kg·d) was obtained here. Multiplied by a factor of 40 gives a rate of 78.7 mg CN/(kg·d) or 3.3 mg CN/(kg-h). A rate of 3.3 mg CN/(kg-h) at 22°C and of 3.4 mg CN/(kg-h) at 24°C, which is obtained if only root mass is considered, is...
in fair agreement with the earlier obtained result of 4.1 mg CN/(kg·h) for weeping willow leaves at room temperature (23°C) and the same initial concentration (Yu et al., 2004). Furthermore, the rate determined here does not correspond to the maximal removal rate \( v_{\text{max}} \), which would be obtained at higher initial concentrations or by a non-linear fit to result from varying concentrations.

In our previous work, the influence of temperature on the removal of cyanide by weeping willow leaves in closed bottles was studied. In the temperature range from 11 to 32°C, a value for \( Q_{10} \) of 2.09 was found (Yu et al., 2005c). This increase in cyanide removal with temperature could be half explained by an increase of the transport kinetics (due to a lower cuticle permeability at higher temperature) and half explained by an increase in the activity of the enzymes that metabolize cyanide.

In this study, experiments were done with intact trees and the uptake of cyanide was by roots. The \( Q_{10} \) value obtained is lower, only 1.46. The variation in temperature affected both the kinetics of loss from solution and the transpiration. Subsequently, the observed change of the loss of cyanide with temperature could be due either to an increased uptake of the compound into root cells (with transpiration water or by diffusion) or to an increase in the activity of the metabolizing enzymes. The log-linear correlation between metabolic rate \( \log v_p \) and temperature is highly significant (Figure 4; \( r^2 = 0.983 \)). The correlation between temperature and transpiration is weaker (\( r^2 = 0.60 \)), but still highly significant (\( \alpha < 0.01 \)). Since humidity and light were kept constant during the experiments, an even better correlation might be expected, but the size of the individual trees varied and had some influence on the correlation between temperature and transpiration. The correlation between transpiration and cyanide loss from the system (assumed to be metabolism) is also significant (\( r^2 = 0.52 \), significant at \( \alpha = 0.05 \)). But a partial correlation between temperature, metabolism, and transpiration unveils that the correlation between temperature and metabolic rate, assuming constant transpiration, would still be 0.96. On the other hand, the partial correlation between metabolism and transpiration (assuming constant temperature) is not significant (\( \alpha = 0.05 \)). It can be concluded that the transpiration as such had no direct influence on the loss of cyanide from solution. Indeed, it was found earlier that the removal of leaves, which stops transpiration almost completely, did not change the removal of cyanide from solution, as compared to intact trees (Larsen et al., 2005). This indicates that cyanide uptake into roots was mainly by diffusion and independent of the uptake of water. However, according to the Einstein relation (Barrow, 1980), a temperature increase from 11°C to 32°C means an increase of the diffusion coefficient by only 6.7%. Therefore, we cannot explain the increase in loss with temperature, which we found in this experiment, with the faster diffusion of the molecules into roots, except if the permeability of the roots would increase greatly with increasing temperature. Subsequently remains as most plausible reason for the increased loss at a higher temperature a higher activity of cyanide-metabolizing enzymes at a higher temperature.

**CONCLUSIONS**

The results of this laboratory study have demonstrated that a large fraction of cyanide was readily taken up and metabolized by weeping willows from the hydroponic solution at all treatment temperatures. Only small amounts of the applied cyanide were recovered from plant tissue. The temperature coefficient, \( Q_{10} \), which is the ratio of metabolism rates at a 10°C difference, was determined for intact weeping willows to be 1.46. The most likely reason for the increase in cyanide removal from the solution with increasing temperature
was an increase in the activity of the metabolizing enzymes. The increase in metabolism was higher than the increase in transpiration. Therefore, no increased accumulation of cyanide and no increase of toxicity with the increasing temperature was found in the temperature range considered here.

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