C-3.1.2 Studies on Critical Loads for Acidic Substances
A Study on Critical Loads for Acidic Substances on Plant
A Dynamic Study on the Effects of Acidic Substances to Physiological Activity of Coniferous Roots

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Abstract The condition around coniferous trees in the soil is becoming gradually acidic when acid rain falls continuously. Nutrient uptake by the roots of coniferous trees could be affected in such environmental change of rooting zone. The experiments of water culture of coniferous seedlings in modified systems were carried out using 2- and, 3-year-old Japanese cedar(*Cryptomeria japonica*), Japanese cypress(*Chamaecyparis obtusa*), and red pine(*Pinus densiflora*) that are the typical Japanese forest trees. Nine major nutrients such as Na^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}, were given in the water culture solution and growth of trees was observed for three years. The aspects of nutrient uptake by these seedlings and the effects of acidity in culture solution were observed. The following results were obtained. 1) Japanese cedar of 50%, Japanese cypress of 30 %, and red pine of 40% in tested seedlings could live for two years. 2) All seedlings,Japanese cedar, cypress, and red pine, that started in the strong acidic condition (pH=3.0) were dead within three months. 3) The minimum pH value in the acidic condition that seedlings can live for two years is estimated as 3.2. 4) The seasonal pattern of the uptake of nitrogen nutrient by Japanese cedar was determined. 5) We offer a hypothesis that coniferous trees have a buffering mechanism against acidification around rooting zone. 6) We found that excess load of nitrate ion in culture solution disturb the avility taking up water from their roots of seedling.

Key Words water culture, Japanese cedar, Japanese cypress, red pine, acidic effects

1. Introduction

When the relationship between the excess load of acidic deposition to the forest soil and the forest decline is discussed, three main explanations have been offered. 1) The cations of important nutrients for the normal growth of forest trees such as K^+, Ca^{2+}, and Mg^{2+} in soil and soil solution are leached from rooting zone by the excess load of acidic substances.[1-3] 2) The toxic cations such as Al^{3+} and Mn^{2+} in the soil solution are concentrated by the soil acidification.[4-5] 3) Excess load of ammonium ion to the forest soil results imbalance of nutrient uptake of forest tree.[6] However, most of these observations were studied in field experiments. It happened that an explanation can be applied on the limited area of forest decline but this explanation is not suitable on another area. It is fact that the tree, as well as other plants, needs nutrient for their life support. Insufficient uptake of nutrient results in dieback and death for all plants. Water culture method of tree seedling is one of the way to get the knowledge about essential and common nature of tree. It is expected to find out what is the factors making strong damages to the seedling for their life support when they forced in the severe environmental conditions through the water culture experiments. It was examined in this experiments that how long the coniferous seedlings could keep their life under terrible environment such as a condition in limited nutrients without essential micro nutrients and in strong acidic solution. The growth aspects of seedlings were observed in relation to the mineral nutrient uptake and the effects of solution acidity. The seedlings of typical Japanese forest trees, Japanese cedar(*Cryptomeria japonica*), Japanese cypress (*Chamaecyparis obtusa*), and red pine (*Pinus densiflora*) were used in experiments.

2.Research Objective

First, it was examined to find out the limiting lowest pH value of culture solution in which the seedlings can live at least for a year. Starting culture solutions with interval of 0.2 from pH 4.0 to 2.8 were prepared and culture experiments using three kinds of seedling were carried out. Second, the long term experiments, for 2 and 3 years, were carried out using culture solutions containing only main essential nutrients, not given any micro essential elements. Third, the nature and characteristic of seedlings for the uptake of each essential nutrient were observed in about 700 times 20-days short term experiments. From these experiments, acidity effects on the growth of seedlings and the relationship between seedling growth and nutrient uptake were considered.

3. Research methods

The experiments were carried out in the green house. The 20 polyethylene tanks of 20 litter volume were setting for water culture of 2-, and 3-year-old Japanese cedar (*Cryptomeria japonica*), Japanese cypress(*Chamaecyparis obtusa*) and red pine (*Pinus densiflora*) The temperature in the green house was not controlled excepted in summer time. The roof of the green house was covered with the sheet made by straw to avoid much difference of the temperatures between inside and outside in summer time. Then, the temperature in the green house was 4 ^{o}C to 8 ^{o}C higher than that of outsides through the year. The coniferous seedlings are planted in the field near the green house and are taken for the culture experiments. At the beginning of the experiment, same aged testing seedlings were planted into the two pots for each seedling with 45 cm diameter and 50 cm of height in the green house. The 80 % of these pots were filled with the forest soil of Tokyo University Forest in Aichi, Akazu No. 32 block, Seto, Aich. The growth of both seedlings in the soil pot and water tank was compared. The air with mini-pump(Fuji 5500) was continuously supplied into the tank through the glass tube with filter. The nutrients were supplied from three basic solutions.

A: NaCl 2g, K_3PO_4 10g, $(NH_4)_2SO_4$ 10g, K_3PO_4 10g, in 3 L

B: $CaCl_2 \cdot 2H_2O$ 10g in 500 mL, C: $MgCl_2 \cdot 6H_2O$ 10g in 300 mL

The 100 ml of A, 10 ml of B, and 5 ml of C were taken into the culture tank and adjusted to 19 litter with distilled water. The concentrations of each nutrient are as follows;

Na^+: 0.060, K^+: 0.419, NH_4^+: 0.264 (~0.500), Ca^{2+}: 0.142 (~0.510), Mg^{2+}: 0.086 (~0.240)
Cl^-: 0.288 (~0.625), NO_3^-: 0.173 (~0.405), PO_4^{3-}: 0.246, SO_4^{2-}: 0.264 (~0.750).

(Ion concentration = $meq \cdot L^{-1}$. Values in parentheses are the concentration range used supplemental experiments)

A culture solution was used for 20 days and then renewed. It renewed within more short time, 7 to 14 days, in spring time. The daily change of all nutrient concentrations of cations and anions in the culture solution was determined by two ion-chromatographic analyzers (Yokogawa Analytical Systems, IC 7000). The pH and the decreased volume of the culture solution were measured without addition of water. The concentration of nutrients in culture solution is changed, but the amount of nutrient taken up by the root can be calculated by data of the decreased volume of culture solution. The growth of seedlings in the culture solution was carefully observed comparing with the seedlings planted in soil pots during three years.

4. Results

The results obtained in this experiment are summarized as follows;

1) The seedlings of 50% Japanese cedar and 30% Japanese cypress of used seedlings in experiments lived for two years within the 7% leaf damage. It means that they succeeded four times shoot growth in spring and autumn with only given nutrients. When essential nutrients such as micro nutrients were not given in culture solution, they probably used efficiently the nutrients originally having in their body before cultivation started. They supported life activity in such away at least for two years. The longest living Japanese cedar in same condition is three and half years with about 30% damage of leaf and still live in the present time.

2) On the other hand, when we cultivated seedlings in the solutions of strong acidic condition, they were all dead within three months. It means that strong acidic condition as pH = 3.0 make much damage against life support of the seedlings and they could not live in such environment. It is concluded from these experiments that the lower limit for the seedlings they can keep activity even in the acidic condition around the root was pH = 3.2. These results were shown in Figure 1.

3) Stepwise uptake of nutrients by the seedlings was observed as shown in Figure 2. The magnitude they required for each nutrient was roughly determined as follows;

$$\underset{(10)}{NH_4^+} > \underset{(5)}{NO_3^-} > \underset{(3)}{Ca^{2+}} > \underset{(2.5)}{Mg^{2+}} > \underset{(2)}{K^+} \approx \underset{(2)}{PO_4^{3-}} > \underset{(0.5)}{Na^+} \approx \underset{(0.5)}{Cl^-} \approx \underset{(0.5)}{SO_4^{2+}}$$

The values in parentheses are roughly determined relative index. These values show the imaginative strength of seedling for the uptake and are determined on the assumption of the index of NH_4^+ being 10. These values were roughly calculated from the consumed amounts of nine ions of about 500 times of experiments during two years. It seems that the seedlings do not want so mach nutrients such as Na^+, Cl^-, and SO_4^{2+}. The turning points were observed on the real concentration lines of Na^+, Cl^-, and SO_4^{2+} ions. It means that the volume of water uptake by seedling increased from this points. This facts obviously related to the concentration of NO_3^- ion in culture solution. When NO_3^- ion was completely taken up into the seedling, water uptake ability of seedling increase. These results suggest that excess load of NO_3^- ion disturb water uptake activity of seedlings.

4) As shown in Figure 3, the seasonal pattern of uptake of nitrogen nutrient was determined. It seems that the seedling required much nitrogen when their shoot grows in the season. The amount of the seedling needed nitrogen as a nutrient was quite different depends on the season in a year. The difference between the minimum and the maximum values was about ten times. The seedlings they succeeded for their two year's life support took up nitrogen nutrients sufficiently. The reason seedling lived for two years or more short time can not withdraw from these experiments, but it looks certain that smooth uptake of nitrogen nutrient is very important activity for their life support.

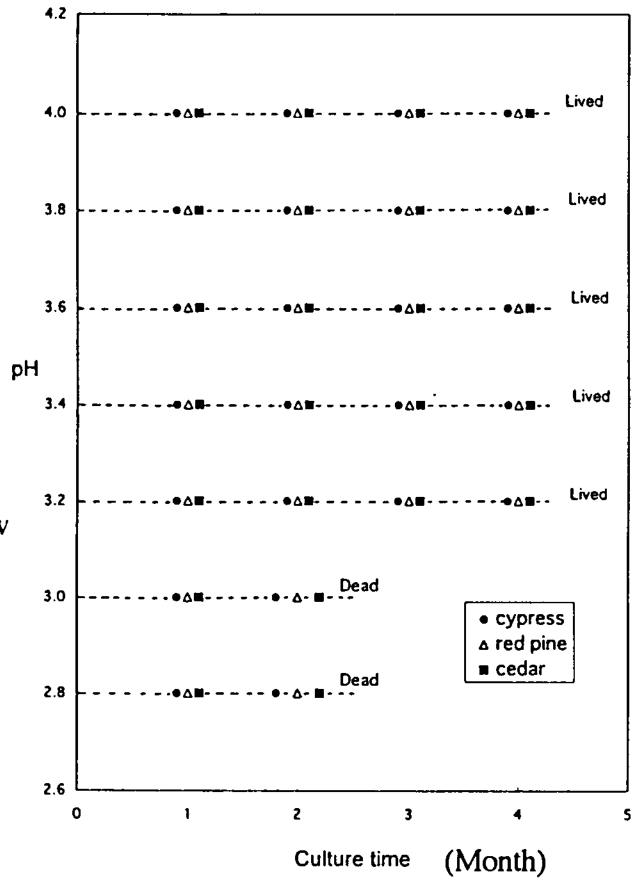
5) The basic data concerning buffering mechanism of tree itself were obtained. The results were shown in Figure 4. At the first stage of the experiment, the culture started in neutral solution around pH 7.2. The seedling seems to prefer such a weak acidic condition as pH 4.6 to 5.0. In 4 hours after culture started, pH value of the solution became stable. In this culture period, H^+ ion in seedling and Ca^{2+} ion in solution have exchanged. At the second stage, strong acid(perchloric acid) was added to the solution. A buffering reaction by seedling was observed at this point. The seedling tried to neutralize the added acid and to change the solution into weak acidic conditions. In consequence of the buffering reaction by seedling, the change of concentrations, that is, Ca^{2+} ion increased and H^+ ion decreased. We presume that the two ions are stored in the free space consisted of cell walls and space between cells at the first layer from root surface and both are in watch-and wait attitude against changes of condition around root of trees.

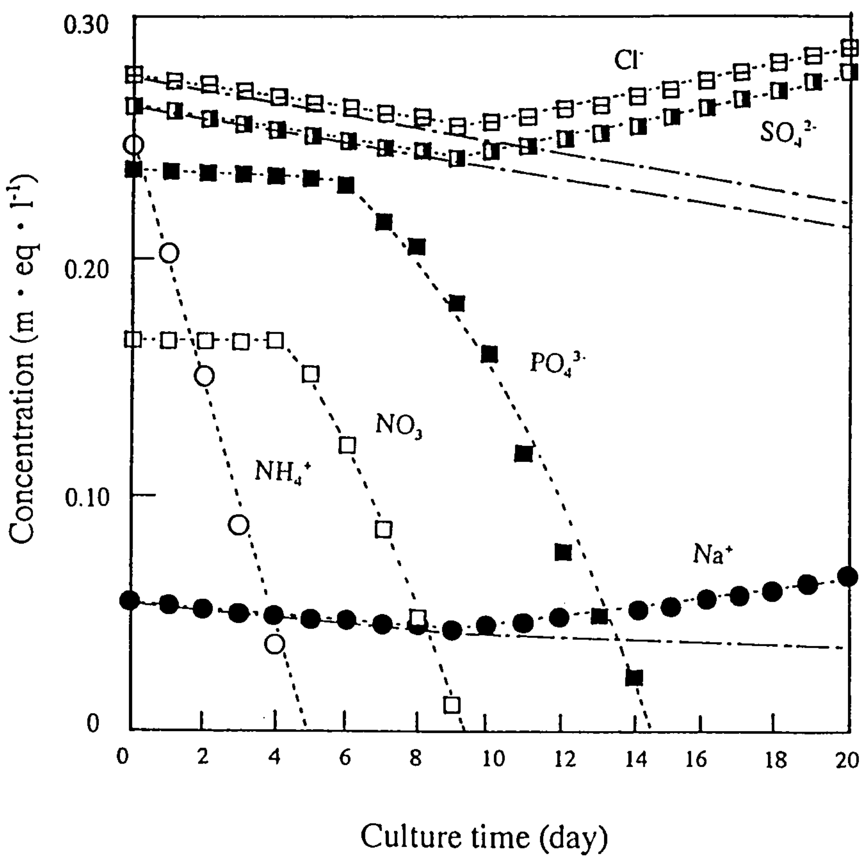
Figure 1. (Right)

The relationship between pH of starting culture solution and growth of seedlings

Figure 2. (Down)

Concentration change of nutrients in culture solution (Chain lines show calculated concentrations with following equation;
C_c(calculated concentration) = C_r(real time concentration) X V_r(real time volume)/ V_s(volume of starting , 19 l), 3-year-old Japanese cedar)





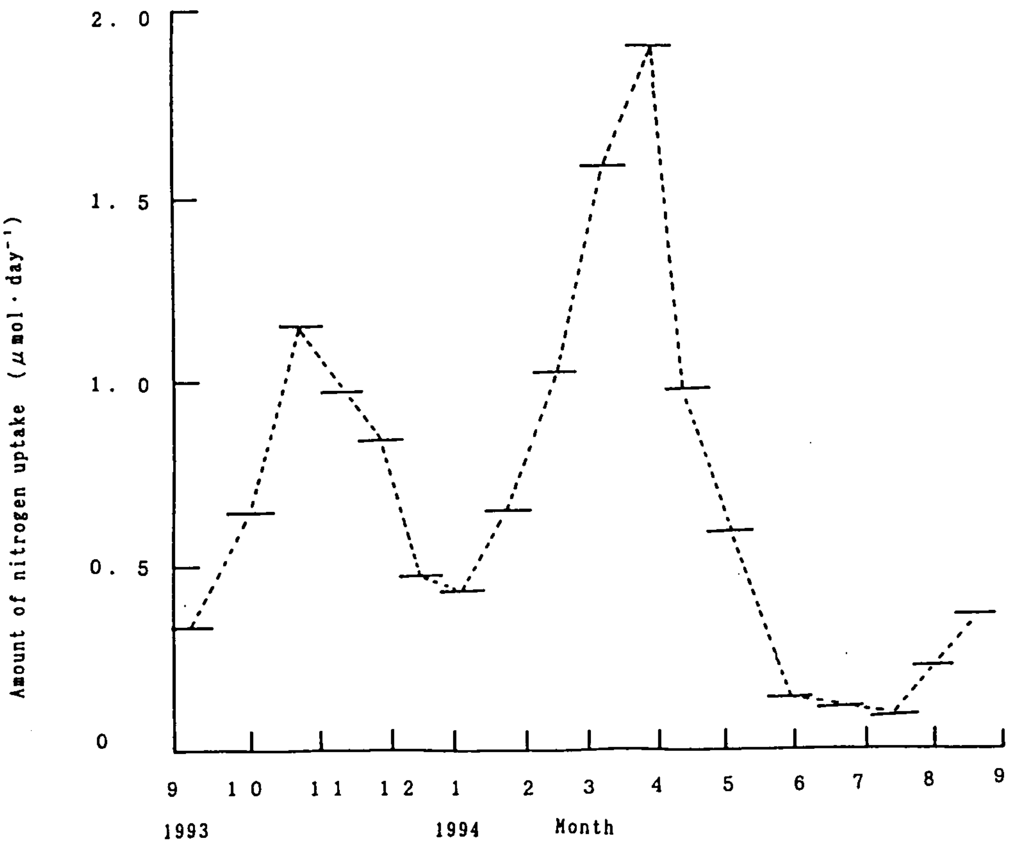


Figure 3. Seasonal change of nitrogen consumption (3-year-old Japanese cedar)

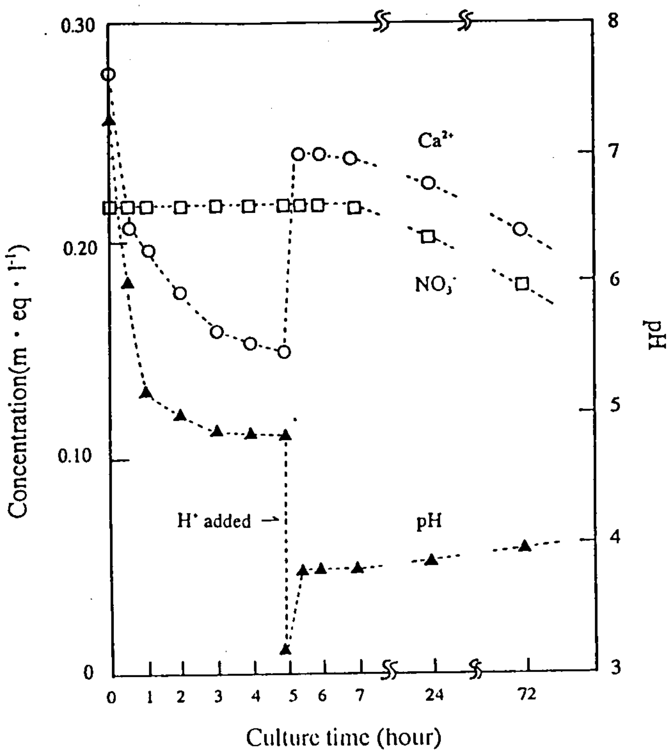


Figure 4. Concentration changes of Ca^{2+} and NO_3^- ions in culture solution and pH change (3-year-old Japanese cedar)

5. Discussion

It seems quite clear that the strong acidic condition as pH=3.0 around the rooting zone makes heavy damage to coniferous trees. All seedlings tested in culture experiments were dead within three months in such strong acidic condition. The minimum pH value in the solutionis seedlings can live at least a year is estimated as 3.2 for Japanese cedar, cypress and red pine.

A critical load based on the H^+ ion concentration around the rooting zone environment is designed for coniferous trees as follows (the concentration of H^+ ion must be below pH = 3.2 (0.63 $mmol \cdot l^{-1}$);

$$\text{Load index}(H_{cl}) = \text{Acid load}(H_l) - \text{Buffering amount by soil}(H_{b1}=H_{soil} - H_{3.2})$$

$$- \text{Buffering amount by soil solution}(H_{b2}=H_{soln} - H_{3.2})$$

$$- \text{Buffering amount by tree}(H_{b3})$$

$H_{cl} = 0$ Critical load, $H_{cl} < 0$ Safety status $H_{cl} > 0$ Dangerous status

H_{b1} can be obtained when forest soil is titrated till pH = 3.2 by acid. H_{b2} can be obtained when soil solution is titrated till pH =3.2 by acid. H_{b3} is determined as 0.062 -0.123 mmol H^+ per dry g root of Japanese cedar, cypress, and red pine in this experiments.

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