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## 論文の内容の要旨

Tama River, originating from the mountains in the western district of Tokyo, exemplifies problems that are typical of Japanese rivers. Except for the downstream reach close to the river's mouth, most of the riparian area of the Tama River was originally stony, covered only with ephemeral annuals. However, a substantial amount of gravels were excavated from the river bed after the World War II. Then upstream Ogochi dam was constructed in 1957. These events have led to a series of changes in the downstream riparian environments. Since the 1960s, the floodplain has gradually become vegetated. Most dominant lianous species was *Sicyos angulatus*. In last few years, many colonies of *S. angulatus* in this floodplain have been replaced by another liana, *Pueraria lobata*. Today, these two species apparently compete and dominance of one suddenly switches to the other. In this study we have investigated the underlying mechanisms of succession and invasion of *Pueraria lobata* and *Sicyos angulatus*. It was hypothesized that hydrological regimes and edaphic factors related to the Tama River floodplain govern the succession and invasion of *P. lobata* and *S. angulatus*. It was also assumed that *P. lobata* and *S. angulatus* might have allelopathic potential which they use as a tool of competition with neighbor species.

To test the above-mentioned hypotheses related to the succession and invasion mechanisms, we observed natural growing populations of *P. lobata* and *S. angulatus* in three sites, viz. Fuchu, Komae and Ohguri, along the bank of Tama River since April 2008 through April 2010. Edaphic and hydrological parameters (soil particle size, soil moisture content, organic matter content, total carbon (TC), Total nitrogen (TN), total phosphorus (TP), K, Ca, Mg, Cu, Zn and total phenolics content of soil) of these sites along with plant parameters viz., aboveground biomass (AGB), belowground biomass (BGB), phenolic contents of *P. lobata* and *S. angulatus* were estimated. A fertilization experiment was conducted in Fuchu and Ohguri to explore the sensitivity of *P. lobata* in its early growth period to N- and P-limitation in soil. Allelopathic phenomenon of *P. lobata* and *S. angulatus* was examined by a series of experiments in laboratory and greenhouse conditions.

In allelopathy study, different organs (leaf, stem, root and seed) of *P. lobata* were extracted with distilled water or methanol. Then those extracts were bioassayed against radish and lettuce seeds to examine the sensitivity of the test species' (radish and lettuce) germination processes. Aqueous and methanol extracts of *P. lobata* organs were assayed for allelopathy using lettuce and radish seeds. Both leaf and root extracts significantly inhibited all of the measured germination indices (total germination, speed of germination and coefficient of the rate of germination) (all  $P < 0.01$ ). When treated with leaf extract, the total germination of both species was ~ 20% less than the control. Furthermore, the leaf extract significantly reduced the speed of germination to 38% and 53% than that of the lettuce and radish controls, respectively. Lettuce and radish seeds soaked in leaf and root extracts for 24 h imbibed less water (~ 30% for both species) than those soaked in distilled water (control), suggesting that a reduction of water imbibition might be one of the mechanisms of germination retardation. Stem and seed extracts affected neither the water uptake nor the germination indices of radish and lettuce seeds. *P. lobata* leaves and roots contain higher amounts of total phenolics ( $P = 0.001$ ) and soluble phenolics ( $P = 0.005$ ) than stems and seeds, consistent with the results of the germination bioassays. In agar plate bioassays, both litter and rhizosphere soil had phytotoxic effects on the radicle growth of radish ( $P = 0.003$ ) and perennial ryegrass ( $P = 0.001$ ) seedlings. Perennial ryegrass (*Lolium perenne*) and cobbler's pegs (*Bidens pilosa*) seedlings grown on leaf and root leachate-amended soil gave ~ 40% shorter roots and shoots and ~ 50% less dry weight than those grown in leachate-free soil.

In another study, the allelopathic effects of *P. lobata* litter on the seed germination and early growth of perennial ryegrass (*Lolium perenne*) and cobbler's pegs (*Bidens pilosa*) was investigated. The bioassays with various concentrations (10, 20, 30, 40 and 50 g L<sup>-1</sup>) of aqueous *Pueraria lobata* litter leachates significantly affected the germination percentages and radicle growth of both species. These parameters decreased progressively when the seedlings were exposed to increasing concentrations of leachates. Root and shoot length, dry weight, and chlorophyll concentrations of *B. pilosa* and *L. perenne* seedlings were also significantly affected when they were grown in leachate-amended soil. However, the chlorophyll fluorescence ( $F_v / F_m$ ) values of seedlings subjected to different concentrations of aqueous *P. lobata* litter leachates indicated that the mere presence of leachates in soil was not stressful for the plants. Seedlings of both species were under stress conditions only when grown in soils treated with higher concentrations of leachates (30, 40 or 50 g L<sup>-1</sup>). A six-week decomposition study of *P. lobata* litter in soil was conducted to observe the retention of phenolic content in soil. The results showed that although the concentration of dissolved organic carbon decreased with increasing decomposition, the phenolic concentration was not significantly affected when the observation period ended and at that point the soluble phenolics in the soil solution were at a level (>150 ppm) considered to be allelopathic. This suggests that the phytotoxic properties of *P. lobata* litter remain stable in soil systems for a considerable time after incorporation into the soil.

Based on the comprehensive bioassay approach and growth study in soil, it was suggested that *P. lobata* has allelopathic effects on its associated species. High concentrations of phenolics in its leaves and roots may play a leading role in allelopathy, which is primarily consummated by retarding seed germination and seedling growth. Though in the present research, the allelopathic phenomenon of *P. lobata* was established, the identification and quantification of responsible phenolics were not done. Therefore, future research should identify and quantify the phenolics produced by *P. lobata* and also determine whether they are phytotoxic alone or in combination.

In allelopathy study, no phytotoxic phenomenon of *Sicyos angulatus* could be detected. Neither aqueous nor the methanol extract of *S. angulatus* organs had significant effects on germination indices of radish and perennial seed in bioassay study. The phenolic contents of its organs were also very negligible. Therefore, it was concluded that *S. angulatus* might not have any allelopathic potentiality.

By physicochemical observations of *P. lobata* and *S. angulatus* in tama River floodplain, it was found that higher soil nutrient concentration favored the biomass production of both species. However, *P. lobata* can thrive very low nutrient habitat due to symbiotic association. *S. angulatus*, on the other hand, grew only in fine sediments with high organic matter, soil moisture and nutrient content. In N-limited soil, though the biomass yield of *Pueraria* was correlated with soil N availability, in N-rich soil, phosphorus is limiting nutrient for it. On the other hand, both N and P was limiting for *S. angulatus*.

Both soil factors and flooding regimes affected the succession and competition of liana species in the Tama River floodplain. Soil factors appeared to be the primary determinant of the pioneering species' colonization. The vegetation history of the sites revealed that in coarse sediments, the pioneering successors were rhizomatous plant species like *Phragmites japonica*, *Miscanthus sacchariflorus* etc. which can assimilate nutrients and moisture form very unfertile soil. Then less tolerant plants like *Pueraria lobata* invade and by its rapid growth habit and vine nature, outcompetes pioneering species. Due to huge annual biomass turnover, *P. lobata* adds a substantial amount of litter into to inhabiting soil and the soil structure becomes finer and the nutrient and moisture condition of the soil also become better. Besides, this species make the soil N-rich by adding atmospheric N into soil with the help of *Rhizobium* bacteria. At this stage less tolerant species like *S. angulatus* invade the place. When both *P. lobata* and *S. angulatus* co-exist in a place, besides soil factors, their competition is dependent on the flooding condition of that site. Due to very shallow and superficial root system, *S. angulatus* cannot stand heavy flood and easily being washed away. *P. lobata*, on the other hand is tolerant to washing away by flooding. However, during moderate flooding, fine sediments trapped by vegetation which favors the growth of both species.

## 論文の審査結果の要旨

河川の生態系は極めて攪乱の多い生態系であり、レキ河原もその例外ではない。そうした場所をハビタートとする攪乱を好む植物にとってレキ河原の生息環境は極めて重要な場所である。ところが、近年、レキ河原の植生バイオマスの増加に伴い、こうした環境は失われつつある。特に、蔓植物の繁茂が多くなり、こうした植物は高い繁殖能力から徐々にレキ河原の環境を消失させてきており、生物多様性保護の観点から大きな問題になってきている。本論文はそうした中で特に増加の著しい、クズと外来種のアレチウリについて、増殖の原因をその機構面から調べたものである。

研究においては、まず、現地実験において、これらの蔓植物の生長律速栄養塩を調べている。実験では、土壤にリンおよび窒素を付加し、付加した場所と付加しない場所の植物の生長量の差から律速栄養塩を調べている。実験の結果、アレチウリの生長には窒素が律速し、アレチウリは高い窒素濃度の下、高い生長量を維持していることが得られている。一方、クズの生長にはリンが律速となっている。この原因として、クズは窒素固定細菌との共生を行い、このために窒素の栄養塩律速を克服すること、また、窒素固定細菌の窒素固定の過程で大量のリンを必要とすることから、これが律の要になっていると考えられた。

次に、これらの植物の優占性を助長する上で、これらの植物のもつアレロパシー性が室内実験および温室における実験で調べられた。まず、アレロパシー物質がフェノール類であることが確かめられ、次にこれによるアレロパシー効果が測定された。実験においては、葉、茎、根等からの抽出液を用いて、これにダイコンおよびレタスの種を浸し、これらの発芽率、発芽速度等が測られた。その結果、アレチウリにおいては、アレロパシー性はみられなかったものの、クズにおいては、葉からの抽出液においては、コントロールと比較して20%程度の発芽率の減少、また、発芽速度においては、レタスで38%、ダイコンで53%の減少がみられ、高いアレロパシー性が観測された。さらに、実際に河川敷において競合の対象になっているネズミホソムギにおいても生長抑制効果が測定され、根や茎においてコントロールと比較し、40%の減少、乾燥重量において50%の減少がみられた。

次に、クズの枯死後蓄積されるリターのネズミホソムギ等に対するアレロパシー効果を10-50g/Lのリター量に対して測られた。その結果、クロロフィルによる蛍光比 ( $F_v/F_m$ ) の値から、30g/L以上のリター量で有意な効果が確認された。以上の結果から、クズが排他的に優占する理由として、クズのもつアレロパシー効果が関与していることが確認された。

次に、実際の河川における減少把握として、多摩川を対象に、クズおよびアレチウリの分布状況、遷移が観測された。ここでは、多摩川の中流部3か所を中心に、クズおよびアレチウリの繁茂状況が調査され、それぞれの特性が得られている。その結果は以下のようなことである。河川の土壤は植物の生長にとっては通常窒素が律速となることが示されるが、クズは比較的貧栄養で侵入可能なものの、アレチウリが侵入するには土壤の高い窒素濃度が必要であり、また、リンも律速となっている。クズが貧栄養で侵入可能な背景には、クズの空中窒素固定細菌との共生する性質がある。また、河川の貧栄養な土壤においては、貧栄養に強いツルヨシやオギの群落が形成されるが、同じく貧栄養に強いクズ群落が発達することで、クズ自体に依る他の群落を排除、アレチウリの侵入を可能にすることで元々の植物の群落を排除する機構が確認された。

論文の構成では、第1章がGeneral Introductionとして河川の草原化等の問題が紹介され、第2章をStudy Sites and Study Speciesとし、ここで対象にしている多摩川に関する水文学的また植生の遷移の状況が記述されている。その後で、第3章でAllelopathy Mechanismとして、本論文の一つの柱であるアレロパシーに関連する部分について記述している。その中では、特に関連する序、目的、過去の文献整理、実験に用いた材料と方法の説明、結果と考察が述べられている。次に第4章で、Physicochemical Mechanismと

して、もう一つの柱である、土壌特性と対象とする蔓植物との関係を、関連する序、目的、過去の文献整理の後で、実験に用いた材料と方法の説明、結果と考察の順に記述している。均整のとれた内容となっている。

Md.Harun Or Rashid の審査に関しては、審査発表会以前に、申請者が各審査員と個別に論文の内容について質疑応答等を行い、概略問題のないことを確認した。その後、審査会を開催し、内容についての40分間の発表の後、質疑応答が行われた。質疑に関しては、様々な視点からの質問が出されたが、概ね、問題なく回答された。

また、語学能力に関しては、論文の内容は英語で記述されていること、いくつかの国際ジャーナルに論文を発表していること等から十分な語学能力を有すると判断された。

なお、本論文に関連する内容は、国際誌：Weed Science(IF=1.451, 70/172, Plant Science) および Weed Biology and Management(IF=0.743, 119/170, Plant Science) に公表しており、基準は満たされている。

以上より、Md.Harun Or Rashid は博士（学術）の学位の資格があると判断された。

以 上