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SUMMARY OF DOCTORAL THESIS

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Title:

Study on Mitigation of Allelopathy and Autotoxicity in Replanting Problem of Asparagus (*Asparagus officinalis* L.)

(アスパラガスの連作障害におけるアレロパシーおよび自家中毒の軽減に関する研究)

Asparagus has a replanting problem regarding of allelopathy when retransplanted new seedlings at the same field. Therefore, we conducted three different experiments to clarify growth and nutrient uptake inhibition and how to recover under replanting conditions. In this study, we evaluated the allelopathic potential and autotoxic interference with growth and nutrient uptake of two asparagus varieties; UC157 (U) and Gijnlim (G) from USA and Europe, respectively. The two varieties were cultivated in a continuous replanting system in different rotational patterns with soil amended by asparagus root residues, and unamended soil (control) under greenhouse conditions. The combinations consisted of: G, U and GU, UG, GG, UU and GUG, GUU, GGU, UGU, UUG, UGG, GGG, UUU for the first planting, first replanting and second replanting, respectively. In this study, UC157 showed higher varietal allelopathic and autotoxic effects as compared to Gijnlim. Further, growth inhibition was correlated with nutrient uptake inhibition; phosphorus (P) uptake was the most inhibited nutrient among nitrogen (N), potassium (K), calcium (Ca), and magnesium (Mg). Contrastingly, UC157 altered soil composition significantly ($P \leq 0.05$) more than Gijnlim after subsequent replanting; for instance pH, C: N ratio and N, P, K, Ca and Mg were decreased whereas electrical conductivity (EC) was increased. Principle component analysis (PCA) of the total variation in different rotational combinations in the inhibitory effect of root residue amendment after the first, second replanting showed the correlation ship within the same replanting treatments among different rotational combination.

Allelopathy research in various aspects has been applied to the fields of agriculture and forestry in order to reduce environmental pollution and increase agricultural production for sustainable agriculture. Allelochemicals are a major reason for replanting problem of asparagus. The effects of potential allelochemicals on the growth of asparagus cultivars UC157 and Gijnlim in replant culture were investigated. Their contents exuded from the roots of each cultivar were determined by HPLC, and their phytotoxicity during the replanting time assessed in agar medium. Organic acids, oxalic, succinic and tartaric, were the main identified allelochemical compounds from the roots of asparagus cultivars. The second replanting produced a higher concentration of total allelochemicals than the first. In general, root and shoot biomass were inhibited by up to 81 and 68%, respectively in the second replanting of UC157. The inhibition of lettuce growth by oxalic, succinic and tartaric acids was also investigated, using a bioassay. The inhibitory concentration (IC_{50}) and the effects of these allelochemicals compounds, oxalic (11 mg l^{-1}), succinic (18 mg l^{-1}) and tartaric (6 mg l^{-1}) acids might have an important function in the allelopathic responses of asparagus replanting problem.

The influence of varietal resistance to allelopathy and autotoxicity for growth, nutrient uptake and allelochemical characteristics were also assessed under laboratory conditions. The two asparagus varieties; UC157 (U) and Gijnlim (G) were cultivated in different rotational patterns in a continuous replanting system. The rotational combinations consisted of: UG, GU, GG, UU and GUG, GGU, GUU, UGU, UUG, UGG, GGG, UUU for the first and second replantings, respectively. The control planting was the first planting of each variety.

The two varieties exhibited significant ($P< 0.05$) differences in growth, nutrient uptake and allelochemical characteristics while UC157 showed more varietal allelopathic and autotoxic activities than Gijnlim after two subsequent replantings. Root and shoot growth were inhibited by up to 77 and 73 %, respectively in the second replanting of UC157 (UUU) in compared to control (first planting of UC157). Growth inhibition was correlated with nutrient uptake inhibition; phosphorus (P) uptake was the most inhibited nutrient among the other nutrients such as nitrogen (N), potassium (K), calcium (Ca), and magnesium (Mg). The identified allelochemicals were oxalic, succinic and tartaric acids in the root exudates of two varieties; whereas UC157 produced the highest concentration of total allelochemicals than Gijnlim; suggesting that allelochemicals could be responsible for both growth and nutrient uptake inhibitions in asparagus cultivation systems. Contrastingly, these inhibitions indicated that selection of suitable asparagus varieties and varietal rotations are necessary in replantings in order to minimize the negative impacts of varietal allelopathy and autotoxicity. The varying degree of inhibition with differential responses to the allelopathic and autotoxic compatibility may be valuable in predicting the potential growth and nutrient uptake inhibitions of subsequent asparagus cultivation. Therefore, we explored the causes of these problems and how to improve growth and nutrient uptake especially for phosphorus (P) uptake under replanting conditions with asparagus varieties. In addition, activated carbon (AC) is widely used technique for neutralizing allelopathic compounds which is secreted or decomposed from root residue (RR). However, this technique also directs effects on plants and soil because it alters growth, nutrient availability, pH and C: N ratio. This study investigated the effect of AC with RR incorporation and P on asparagus growth, nutrient uptake under controlled environmental conditions. AC was incorporated into sandy soil and was amended with 15 years old RR of UC157 before the sowing of asparagus for the first, second and third time of continuous planting. In all the planting, P was applied at P0 (0), P1 (7.5), P2 (15.5) and P3 (22.5) mg l^{-1}, asparagus seedlings growth and nutrient uptake was measured and data shown after third planting stage, caused maximum growth and nutrient uptake reduction. In the absence of RR, AC significantly increased plant growth and nutrient uptake, but when RR was incorporated decreased plant growth and nutrient uptake in all three planting. When AC was incorporated into the soil as phosphorus (P3) increased level and without RR, asparagus root (80 %) and shoot (84 %) biomass increased, total nitrogen (N), phosphorus (P), potassium (K), uptake by 87, 82 and 76 %, respectively. The increased ($P < 0.05$) growth corresponded to increased plant P content, likely resulting from greater P availability. The difference between plant growth in medium with and without activated carbon and in the presence of the potentially allelopathic RR; however, this difference may be biased if AC with applied P alters soil nutrient availability and plant growth even in the presence of the RR allelopathic agent. RR incorporation, however, retarded the effects of fertilization on asparagus growth and P uptake. The combined application of AC and P increased asparagus growth and nutrient uptake. In this study, plant growth and P uptake was increased with the increasing of level of P, but it was not known the optimum level of P, until which level growth will be retarded, therefore, the present steps are currently validating to explore the exact quantity or level of P and to find out the mechanism and specific causes of these problems and how to improve the growth and P uptake under continuous replanting.

In conclusion, these studies provided additional evidence of asparagus varietal allelopathy and autotoxicity in different rotational combinations under greenhouse and laboratory conditions. The growth inhibition of asparagus could be mitigated by proper selection of varieties to reduce the persistence of autotoxins and accumulation of allelochemicals from root residues during replanting. AC and P application will be required under replanted soil to replenish the allelopathic effects of RR incorporation, or to improve growth and nutrient uptake, by overcoming nutrient immobilization resulting from RR amendment in asparagus replanting.