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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：　Study on allelopathy of four invasive plant species （4種の侵略的植物種のアレロパシーに関する研究）
Title of Dissertation

学位論文要約：
Dissertation Summary

General introduction

Allelopathy is a biological phenomenon that plants/microorganisms produce and release certain chemicals into environment and inhibit or stimulate growth of other neighboring plants/microorganisms (Rice 1984; Fujii 2000; Reigosa *et al*. 2006). Chemicals that mediate allelopathic phenomenon are called allelochemicals (in this dissertation, also called allelopathic substances). The releasing pathway of allelopathic substances into environment are such as volatilization from leaves, leaching from leaves by rain drops, exudation from roots, decomposition of plant residues (Rice 1984; Fujii 2000; Reigosa *et al*. 2006). Some released allelopathic substances can be transferred to target plants by mycorrhizal fungi (Achatz *et al.* 2014).

Invasive plant species are the non-native plant species that come from original habitats to new areas over long distance, aggressively spread there, and damage to environment, biodiversity, economy, and human health (Richardson *et al.* 2000; Hagan *et al.* 2013; DCNR 2018). Richardson *et al.* (2000) mentioned that there are three steps for invasion. First is introduction phase where invasive plants break in to new areas beyond geographical range and establish populations of adult plants. Second is colonization phase where the plants in the populations form a colony that is self-perpetuating through reproducing and increasing in number. Third is naturalization phase where those plants spread widely, establish new self-perpetuating populations and becomes incorporated in the native species flora. After such process, invasive plants become serious problems that they often eliminate native plants and form huge monotypic stands, resulting in serious damage on native plant ecosystems.

To understand characteristics of invasive plant species, an important thing is to investigate their allelopathic potential which is suggested to be one of potential factors for successful invasion. The four invasive plant species cogongrass (*Imperata cylindrica* (L.) Raeusch.), Mexican sunflower (*Tithonia diversifolia* (Hermsl.)), Chinese violet (*Asystasia gangetica* (L.) T. Anderson) and burdock (*Arctium lappa* L.) were selected for this study. No or limited information was available on allelopathic potential of these species. Objectives of this study were following.

1. To evaluate allelopathic potential of the four invasive plants cogongrass, Mexican sunflower, Chinese violet, and burdock.
2. To isolate and identify allelopathic substances from those four plants.
3. To determine biological activity of the isolated substances on test plants.

Plant growth inhibitory activity and active substances with allelopathic potential of cogongrass (*Imperata cylindrica*) rhizome

The extracts of cogongrass rhizomes had growth inhibitory activity on the roots and shoots of the six test plants

barnyard grass, ryegrass, timothy, cress, lettuce, and alfalfa, suggesting that cogongrass rhizomes may have allelopathic potential. As the rhizome biomass of cogongrass is large (Tominaga *et al*. 1989), the allelopathy may play an important role in cogongrass dominated habitats. Rhizomes of certain plants contain some plant growth inhibitory substances (Inoue *et al.* 1992; Goto *et al.* 2001; Xuan *et al.* 2009), and some of the plants aggressively extent their rhizomes under the ground (MacDonald 2004; Weston *et al.* 2005). Therefore, rhizomes may be potential parts to provide plant growth inhibitory substances.

After purification of the extracts of cogongrass rhizomes, the four substances, 5-methoxyflavone, 5,2'-dimethoxyflavone, methyl caffeate, and abscisic acid were isolated. These substances were found through a series of separations in which the fractions having higher growth inhibitory activity were further processed. Thus, the four substances may be the ones that can contribute to the plant growth inhibitory activity of the extracts of cogongrass rhizomes. 5-Methoxyflavone, 5,2'-dimethoxyflavone, and methyl caffeate significantly inhibited the root and shoot growth of cress (their I_{50} values were 0.079–0.24, 0.23–1.1, and 0.59–0.88 mM, respectively).

Phytotoxic property of the invasive plant *Tithonia diversifolia* and a phytotoxic substance

Aqueous methanol extracts of an invasive species, *T. diversifolia* inhibited growth of three weed species, *L. multiflorum*, *P. pratense* and *E. crus-galli*, and a crop species, *L. sativum*. It was reported that *T. diversifolia* extracts had growth inhibitory activity on several crop plant species (Tongma *et al.* 2001; Taiwo and Makide 2005; Oyerrinde *et al.* 2009; Otusanya and Ilori 2012; Miranda *et al.* 2015). The present results suggest that *T. diversifolia* extracts also have inhibitory activity on the weed plants.

After purification of phytotoxic substance in the extracts of *T. diversifolia*, a potent phytotoxic substance was isolated and identified as tagitinin C. Tagitinin C has so far been isolated only from *T. diversifolia* (Pal *et al.* 1976; Baruah *et al.* 1979) and the substance was reported to have antiplasmodial, antiproliferative, antiglioblastoma cytotoxic and insect feeding deterrent activities (Goffin *et al.* 2002; Gu *et al.* 2002; Lee *et al.* 2011; Liao *et al.* 2013). Tagitinin C was also reported to have growth inhibitory activity against radish, cucumber, tomato and onion (Baruah *et al.* 1994; Miranda *et al*. 2015). However, its inhibitory activity on weed plant species was not clear. The phytotoxic activity of tagitinin C on *L. multiflorum*, *P. pratense*, *E. crus-galli* and *L. sativum* was evaluated. Tagitinin C was active at concentrations greater than 0.1-0.3 mM and IC_{50} values of tagitinin C were 0.126 - 0.834 mM.

Phytotoxic activity of Chinese violet (*Asystasia gangetica* (L.) T. anderson) and two phytotoxic substances

The leaf extracts of Chinese violet inhibited the growth of the six test plants, suggesting that the extracts have phytotoxic activity and contain some phytotoxic substances. I_{50} values of the extracts of Chinese violet on the test plants were 1.64–143 mg dry weight equivalent extract mL^{-1}. Several biological activities of the extracts of Chinese violet and phytotoxic activity of leaf litter leachate of the plant have been reported (Akah *et al*. 2003; Reddy *et al*. 2010; Nurul *et al*. 2017).

Two phytotoxic substances, indole-3-carboxaldehyde and (6*R*,9*S*)-3-oxo-*α*-ionol, were isolated from the extracts of Chinese violet leaves. (6*R*,9*S*)-3-oxo-*α*-ionol was previously isolated from *Brachiaria brizantha* (Kato-Noguchi *et al*. 2014a; Kobayashi & Kato-Noguchi 2015). The other substance indole-3-carboxaldehyde was previously isolated from several plants such as rice and pea (Rimando *et al*. 2001; Nakajima *et al*. 2002). The substance was also isolated from the pathogens (Maddau *et al*. 2011).

(6*R*,9*S*)-3-oxo-*α*-ionol was reported to have strong phytotoxic activity (its I_{50} values on cress roots and shoots were 25.1–41.7 μ mol L^{-1}) (Kato-Noguchi *et al*. 2014a; Kobayashi & Kato-Noguchi 2015). While, indole-3-carboxaldehyde showed significant inhibition on the root and shoot growth of cress (I_{50} values were 127–210 μ mol L^{-1}). The growth inhibition was concentration-dependent but the pH values were not affected by the substance concentration, suggesting that pH was not a significant factor for this growth inhibition. In addition

to above activity, this substance was reported to cause necrotic lesions on the leaves of oaks, *Quercus suber* and *Quercus ilex* and to inhibit the lateral bud growth of pea (Nakajima *et al*. 2002; Maddau *et al*. 2011). Our study indicates that Chinese violet possesses phytotoxic activity and contains the two phytotoxic substances, indole-3-carboxaldehyde and (6*R*,9*S*)-3-oxo-*α*-ionol.

Phytotoxic activity and an active substance of burdock

The extracts of burdock leaves and roots inhibited the shoot and root growth of cress and barnyard grass where the inhibition levels increased with increasing concentrations of the extracts. This indicates that those extracts contained phytotoxic substances. Based on the I_{50} values, the burdock leaf extracts had 2.0-2.5 times higher phytotoxic activity than the root extracts.

Since the burdock leaf extracts showed higher activity than the root extract, we conducted isolation and identification of an active substance from the leaf extracts. The extracts were purified by a series of bioassay-guided separations and a phytotoxic substance was isolated. The substance was identified as onopordopicrin by comparing spectra data with that in the literature (Lonergan *et al*. 1992) (Fig. 5.7). Onopordopicrin significantly inhibited the shoot and root growth of cress at concentrations ≥ 0.3 mM and those of barnyard grass at concentrations ≥ 1 and 0.3 mM, respectively. In this study, we showed the activity on growth of a test plant cress used often in previous studies and a major weed barnyard grass. Evaluating I_{50} values of burdock leaf extracts and onopordopicrin and its concentration in the leaves, the substance may play a major role in the phytotoxic activity of burdock leaves.

General discussion

In this study, allelopathic potential of the four invasive plant species were evaluated and their putative allelopathic substances were isolated and identified. The four selected invasive plants were cogongrass (Poaceae), Mexican sunflower (Asteraceae), Chinese violet (Acanthaceae), and burdock (Asteraceae).

The extracts obtained from the all four invasive plants cogongrass, Mexican sunflower, Chinese violet, and burdock inhibited growth of the test plants including dicotyledons and monocotyledons concentration-dependently. Plant growth inhibitory activity of the extracts of Chinese violet and burdock was indicated for the first time. Based on their I_{50} values, differences in level of their activity were observed. The extracts obtained from leaves of Mexican sunflower, Chinese violet, and burdock had high plant growth inhibitory activity and less than 0.15 g (in dry weight) of plant materials have potential to inhibit test plant growth by 50% that of control. Leaf is an important resource that provides allelopathic substances through volatilization, leaching by rain drops, and decomposition of dropped leaf litter (Rice 1984; Fujii 2000; Reigosa *et al*. 2006). While, the extracts obtained from cogongrass rhizomes showed a little less activity than others. However, because cogongrass rhizomes spread throughout underground in high density and can release secondary metabolites (MacDonald 2004; Tominaga *et al.* 2007; Xuan *et al.* 2009; Hagan *et al.* 2013), it may be easy to deriver emitted allelopathic substances to neighboring target plants in its stands. Considering above, the all four invasive plant species may have allelopathic potential.

After the bioassay-guided separations, the eight putative allelopathic substances were isolated. The isolated putative allelopathic substances were 5-methoxy flavone, 5,2’-dimethoxy flavone, methyl caffeate, and abscisic acid for cogongrass, tagitinin C for Mexican sunflower, indole-3-carboxaldehyde and (6*R*,9*S*)-3-oxo-*α*-ionol for Chinese violet, and onopordopicrin for burdock. The four invasive plant species had different putative allelopathic substances. The all isolated substances significantly inhibited growth of the test plants concentration-dependently (Kato-Noguchi *et al*. 2014a, 2016). Based on the I_{50} values, abscisic acid had the highest growth inhibitory activity followed by (6*R*,9*S*)-3-oxo-*α*-ionol, 5-methoxy flavone, and indole-3-carboxaldehyde. 5,2’-dimethoxyflovone had the least activity on test plant roots, while methyl caffeate

was the least on test plant shoots.

There are characteristics of invasive plant species considered to be important for their successful invasion. High reproduction and high growth rates, and phenotypic plasticity of the invasive plants may be important for the domination in the new habitats (Thompson *et al.* 1991; Mack 1996; Cappuccino & Arnason 2006). High defense capacity of the plants against pathogens and herbivores may be necessary in the new habitats (Keane & Crawley 2002; Mitchell & Power 2003; Cappuccino & Carpenter 2005). The interaction of the invasive plants with native plants may also be crucial. Some invasive plants have many substances with allelopathic activities, which are highly toxic to native plants (Callaway & Ridenour 2004; Chengxu *et al*. 2011; Meiners *et al.* 2012). Allelopathy has been suggested to contribute to successful invasion and to forming monotypic stands (Hierro & Callaway 2003; Inderjit *et al*. 2008). Since the four invasive plant species cogongrass, Mexican sunflower, Chinese violet, and burdock are also suggested to have allelopathic potential by the present research, there is potentiality that their allelopathic potential may contribute their invasiveness.

In conclusion, the bioassay results of the extracts obtained from the four invasive plant species cogongrass, Mexican sunflower, Chinese violet, and burdock indicated their inhibitory activity on plant growth, suggesting that those invasive plants may have allelopathic potential. Bioassay-guided separations of those extracts resulted in isolation of the eight putative allelopathic substances 5-methoxy flavone, 5,2'-dimethoxy flavone, methyl caffeate, abscisic acid from cogongrass, tagitinin C from Mexican sunflower, indole-3-carboxaldehyde and (6*R*,9*S*)-3-oxo-*α*-ionol from Chinese violet, and onopordopicrin from burdock. Allelopathic potential of those substances were indicated with the I_{50} values ranged from 0.31 μM to 1.1 mM. Those substances may be released into environment and may play an important role in allelopathy. Their allelopathic potential may be involved in their invasiveness and help to successful invasion by suppressing growth of neighboring plant species.

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