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Effects of environmental factors on host plant and its specialist herbivore, *Aristolochia contorta* and *Sericinus montela*

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박 현 준

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Abstract

It is quite obvious that the future biodiversity, including entomofauna, would be at risk by rapid environmental changes as climate change progresses. Considering the ecological and biological values in the ecosystem, the attempt to understand the plant responses to climate change is preferentially needed to be examined because of its significant consequences on insect family. Although there have been many researches, the effects of climate change on plant-herbivore interactions in the context of cascading effects from plants to herbivores are still unclear. In particular, the significance of increased precipitation frequency and the seasonality of plant and herbivore have not received sufficient attention yet.

Here, I tried to address these gaps by conducting three major researches using a native plant (*Aristolochia contorta*) and its specialist herbivore (*Sericinus montela*). First, a field survey in natural habitat of *A. contorta* was conducted to investigate possible effects of various environmental factors on the host plant. Second, I performed two mesocosm experiments using open-top chambers (OTCs) to examine the effects of elevated CO_2 and increased precipitation frequency on plant growth and defenses and consequences to specialist herbivores, and to figure out the seasonality of those effects of two environmental factors. I observed the growth of plant based on stem length and leaf number, and measured the relative growth rate (RGR) of herbivores to assess the cascading effects of plant responses to herbivores' growth performance. I further investigated C: N ratio and primary metabolites as parameters of nutrient value, and

analyzed secondary metabolites as parameters of plant chemical defenses.

According to field survey, the growth period of *A. contorta* could be affected by various biotic and abiotic factors, particularly herbivorous and interspecific competitive stress, and cations in soil. In addition, elevated CO_2 impeded growth with decreased photosynthesis ability, and increased resistance in plants. In contrast, increased precipitation frequency partly ameliorated the negative effects of high CO_2. Growth performance of specialist herbivore decreased under elevated CO_2 condition as a consequence of increased resistance in plants. Furthermore, elevated CO_2 and increased precipitation frequency had different effects on nutrient value and constitutive defenses of host plant in distinct temporal variations. That is, positive effects of increased precipitation on nutrient value were significant in the middle of plant growing season, whereas negative effects of elevated CO_2 on both of nutrient value and constitutive defenses were remarkable in the late of growing season. The unconformable variations of food quality seemed to be responsible for the seasonality of specialist and generalist herbivore.

In conclusion, this research suggests both the quantity and quality of host plants would decline because of significant CO_2 effects, and the growth performance of its specialist herbivore might be threatened as climate change progresses. That is, different scenario but the same predictions of climate change effects on entomofauna is suggested. Nevertheless, considering the seasonality of effects of elevated CO_2 and increased precipitation frequency, less danger of herbivorous insect may be expected because of the ameliorating effect of increased precipitation frequency to

high CO_2 at a certain emergence timing in their life cycle. Additionally, the findings of this research can contribute to enable comprehensive understanding of climate change effects on plant-herbivore interaction, with the consideration of significant variable environmental factor under climate change and species-specific characteristics. This study also highlighted the ecological implications of seasonal dynamics for precise of future plant-herbivore interaction under climate change.

Keyword: climate change, leaf nutrient value, plant constitutive defense, plant-herbivore interaction, seasonality, specialist herbivore

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Chapter 1. Introduction

1.1. Plant-herbivore interaction and secondary metabolites

Plants have various types of biotic and abiotic stressors (Weis and Abrahamson, 1985). Among those stressors, herbivore occupying large portion of animal groups in the whole ecosystem, is one of the most important biotic factors having considerable relationship with plants (Agrawal and Fishbein, 2006). In order to deal with this stressor, plants have developed sophisticated defensive mechanisms in various ways (War et al., 2012). The defensive mechanisms of plants can be briefly classified into two groups: physical and chemical defenses (Feeny, 1976; War et al., 2012). Detail mechanisms and probable situation could be different, but the ultimate purpose of those defenses is lower herbivores' growth and also their fecundity (Rosenthal and Berenbaum, 2012). There are several mechanisms in chemical defenses, nonetheless, secondary metabolites of plants play important roles for plant chemical defenses (Mazid et al., 2011).

Plant secondary metabolites such as nitrogen compounds and volatiles, had been considered as wastes of other metabolisms in the first time, when their functions were obscure (Hartmann, 2007). Several methodologies including gas chromatography (GC) and liquid chromatography (LC) were developed and tested to extract and identify these metabolites (Wink, 1999), and their chemical properties and practical functions for human had been mainly considered in the beginning of researches (Hartmann, 2007). However, they received more significant attention after disclosure of some

roles of them (Theis and Lerdau, 2003; Wink, 2003). Most plant secondary metabolites are revealed as the key compounds for interaction with other plants and animals (Jamieson et al. 2012). Plants can attract beneficial animals such as pollinators and natural enemies for herbivores with volatiles, and also can interrupt other nearby plants' growth through allelopathic ways (Trowbridge, 2014). Among these various important roles in the perspective of chemical ecology, the defensive functions are largely examined because of the responsibility of coevolutionary roles with herbivores (Freeland, 1991; Cornell and Hawkins, 2003). Green leaf volatiles (GLVs), which are largely known as typical plant volatiles, have functions as weapons for pathogens and herbivores and food indicators for herbivores also (Scala et al., 2013; ul Hassan et al., 2015). Other volatiles can directly repel insects attacking them or attract natural enemies of attackers to remove them (Paré and Tumlinson, 1999; Wu and Baldwin, 2010). Through these mechanisms, plants perform rapid and immediate defenses to herbivore.

Besides volatiles, plants also synthesize nitrogen-containing secondary metabolites, which have defensive functions in plants (Miyagawa, 2009). Those metabolites generally are noticeable for inducible and constitutive defenses of plants particularly (Anulika et al., 2016). These metabolites act as deterrence, antifeedant, toxicity, or precursors to physical defense systems even (Singh, 2018). Hence, rather than volatiles, nitrogen-containing secondary metabolites are more considered as a key factor for plants involving relationship with other organisms including herbivores (Hartmann, 2004; Schwachtje and Baldwin, 2008). That is, consideration of nitrogen-containing secondary metabolites is of importance to understand

plant-herbivore interactions thoroughly.

As plants have evolved their own defensive mechanisms, herbivores including herbivorous insects also advanced adaptive mechanisms to overcome plants' resistance (Futuyma and Agrawal, 2009). In other words, herbivores had to develop detoxifying toxic secondary metabolites to benefit individual performance as well as high reproductive output (Ivie et al., 1983). The adaptive mechanisms, however, demand lots of energy to herbivores (Ali and Agrawal, 2012; Gong and Zhang, 2014), so that the range of possible host plants for a certain insect could be narrowed down, becoming specialist herbivore (Berenbaum et al., 1989; Ali and Agrawal, 2012). In this context, this arms race between plant and herbivore is occasionally considered as the potential force of diverse insect community (Clayton et al., 2015; Maron et al., 2019). Contrary to specialist herbivore, some insects called as generalist herbivore have broad dietary spectrum including more than one plant family (Bernays et al., 1994). Although these insects mostly exhibit less growth performance compared to specialized herbivorous insects, it is highly possible that competitive stress on specialist herbivore occur by generalist herbivore sharing the same host plant (van Velzen and Etienne, 2013). Nevertheless, since plants' defensive secondary metabolites can affect not only the performance of specialist and generalist herbivore each but also the interaction between them (Ali and Agrawal, 2012), secondary metabolites of plants are fairly thought to be inextricable for both plant-herbivore and herbivore-herbivore interaction.

1.2. Climate change and plant-herbivore interaction

Global climate change has been progressed due to increasing anthropogenic emission of carbon dioxide (CO_2) after industrialization (Rosenzweig and Hillel 1998; Morison and Lawlor 1999). As the global climate change progress, various environmental factors could be varied in complex ways (IPCC, 2001). This environmental change affects ecosystem in various level from organism to whole ecosystem, including the interactions between organisms (Bellard et al., 2012). Interests of publics as well as researchers in climate change got increased, because the speed of the environmental change had accelerated and the consequent variations in ecosystem had extended (IPCC, 2014). Accordingly, numbers of researches were conducted in the context of climate change and its effects on plant and/or insect community (Coley, 1998; Parmesan, 2006; DeLucia et al., 2012). For those researches, chambers were utilized in the beginning, but open-top chamber (OTC) and free air CO_2 enrichment (FACE) were broadly used after being aware of the possibility of unexpected effect caused by closed condition in chambers (Norris et al., 1996; McLeod and Long, 1999, Piva et al., 2013). FACE experiment, however, could not be easily conducted since it requires high cost and spaces (Kimball et al., 1997). Thus, although FACE experiment can reflect nature the most, OTC has become the most essential and effective experimental method in recent climate change researches which secures both relevancy and reliability enough (Messerli et al., 2015).

With these methodologies, researchers examined the effects of several

environmental factors which are expected to be varied under climate change (Smith et al., 1992; Liancourt et al., 2013). Among various environmental factors, it is clear that the CO_2 concentration will increase and its range will be from 420 to 940 ppm depending on the efficiency of global environmental policies against climate change (IPCC, 2007). Additionally, temperature elevation will certainly be accompanied with the CO_2 elevation because of its greenhouse effect (IPCC, 2007). Thereby, many researches focusing on the effects of elevated CO_2 and temperature on plants as well as insects have been performed in the context of climate change (Lincoln et al., 1993; Pritchard et al., 2001; Kingsolver et al., 2011). As the photosynthesis of plants are one of the most significant biological activity in ecosystem, elevated CO_2 received more attention than high temperature related to the productivity and development of plant communities (Veteli et al., 2002; de Souza et al., 2008). A large number of studies predict the enhancement of photosynthesis under elevated CO_2 condition, called as carbon fertilization, because present CO_2 concentration is insufficient to exhibit the maximum rate of photosynthesis both in C_3 and C_4 plants (Ainsworth and Rogers, 2007). As a consequence, increase of carbohydrates content and decrease of protein and/or amino acids content would be observed (Nie et al., 1995; Taub et al., 2008). Based on those researches, ‘nutrient dilution hypothesis’ was suggested as a noticeable change in plant community (Welti et al., 2020), and as a crucial influential factor to herbivore community also (Pennisi, 2020). Nevertheless, inhibitory effects of elevated CO_2 on photosynthesis activity had been suggested in the beginning of researches about plant acclimation to high CO_2 level (DeLucia et al., 1985), which is not examined enough until nowadays.

Indeed, some researches suggested impeding effect on Rubisco content, photosynthesis activity, chlorophyll regeneration (Sage et al., 1989; Arp, 1991; Bowes, 1991), and a recent research have reported non-fertilizing effect of high CO_2 even (Johnson et al., 2020). Therefore, it is still necessary to examine the effect of elevated CO_2 concentration on plant, particularly considering photosynthetic characteristics.

In the case of insects, direct effects were found in increased temperature (Bale et al., 2002). Their growth increases and their phenology get faster under high temperature condition, because of accelerated physiological metabolism in larvae and pupae (Kingslover et al., 2011). Additionally, indirect effects on high temperature can also be observed in the context of phenological synchrony between host plant and its herbivore (Lee et al., 2016; Ren et al., 2020). For instance, the possibility of temporal mismatch of emergence in Edith's checkerspot butterfly and its host plant is largely known as a result of independent changes in phenological traits of plant and insect under climate change (Singer and Parmesan, 2010; Donoso et al., 2016). In contrast, rather elevated CO_2 mostly have indirect effects on herbivore performance through plant responses (Lincoln et al., 1993; Knepp et al., 2005). For larvae performance, diluted nutritious compounds in plants by improved photosynthesis with high CO_2, representing impeded nutrient value, show decline of herbivore growth (Goverde et al., 2002; Schädler et al., 2007). Moreover, alteration in secondary metabolites under increased CO_2, indicating differed plant resistance, results variations in herbivore growth (Johnson and Hartley, 2017; Xu et al., 2019). Given these facts, the effects of climate change on insects, particularly herbivores having close

relationship with plants, are needed to be examined under the consideration of cascading effects from plants.

Although much of the theoretical and experimental researches have investigated two major changeable environmental factors, elevation of CO_2 and temperature, attempts to focus on other potentially influential factors for plant and insect community such as precipitation are increasing nowadays (Jamieson et al., 2012; Broughton et al., 2017). In fact, due to temperature elevation and consequent variations in evapotranspiration pattern caused by high CO_2, precipitation patterns also differ according to its regional characteristics (Dore, 2005; Pfahl et al., 2017). Some regions are predicted to have longer drought period because of increased intensity but decreased frequency of precipitation (Trenberth, 2011). Mostly, drought condition act as a constraint for plant photosynthesis, resulting the offset of carbon fertilization effect from elevated CO_2 (Albert et al., 2011; Lahive et al., 2018). Contrastingly, according to recent researches, rather increased precipitation frequency is thought to be possible such as in monsoon climate regions (Cha et al., 2016; Myhre et al., 2019). More frequent precipitation alters soil water regime, which would have remarkable effects on plant growth performance and physiological characteristics. In particular, the effects might be more significant for plants inhabiting dry soil condition, since they are usually vulnerable to water-saturated and/or water-logged soil (Keddy, 2017). Hence, consideration of increased precipitation frequency in climate change research is necessary to deepen the understanding of its effects on plant-herbivore interaction, particular for plants whose habitats have dry soil almost.

In addition to environmental factors, interests in the timing of plant-

herbivore interaction have risen. In fact, appropriate agreement in plant and insect phenology is important for natural interactions between them (Elzinga et al., 2007; Yang and Rudolf, 2010) Their phenological traits, however, are independent with each other, resulting different variations caused by climate change (Singer and Parmesan, 2010). Thus, a number of researches suggest the crisis for herbivore as a result of mismatch with its food plant (Yang and Rudolf, 2010; Ren et al., 2020). Nevertheless, focusing on the properties of initial life cycle, such as emergence timing, would have problems of precise understanding in the perspective of long-term responses of ecosystem (CaraDonna et al., 2014; Dorji et al., 2020). Indeed, the emergence of Lepidoptera occur several times a year (Singer and Parmesan, 2010), indicating that plant-herbivore interaction can happen various growth stages of plant. Since plant physiological responses to environments are different according to its growth stage (Yang et al., 2020), the indirect effects of climate change on plant-herbivore interaction through plants would be observed in different ways. Therefore, examination of the seasonality of plant-herbivore interaction under climate change should be performed for more reliable prediction of future ecosystem changes.

1.3. *Aristolochia contorta* and *Sericinus montela* as experimental models for plant-herbivore interaction

Aristolochia contorta Bunge, one of the native plants in South Korea, is a perennial vine plant having its specialist herbivore, *Sericinus montela* Gray (Fig. 1-1). This plant is mostly distributed in East Asia (Nakonechnaya et al.,

2012), and it inhabits waterside region such as riverside and edge of paddy field (Lee, 2003). New shoots of *A. contorta* from root buds generally start to emerge from the early April, while seeds of *A. contorta* usually germinate in late June and July (Lee, 2003). Growth of *A. contorta* is the largest in July and August, and it almost stops in September. There are few researches providing detail information of *A. contorta* habitat, but their locations are known to show low range of soil water content (Park et al., 2019), which is thought to be sensitive to water-saturated soil formed by increased precipitation frequency. Furthermore, it synthesizes toxic nitrogen-containing secondary metabolites, including aristolochic acids (Cheung et al., 2006), and the synthesis may differ according to its surrounding environment (Prinsloo and Nogemane, 2018). Additionally, *S. montela*, which use *A. contorta* as the only food plant, has the importance as vulnerable species (VU) in the red book. The emergence of *S. montela* are known as three times a year, from the middle of April to the early June (Spring type), from the late June to the late July (Summer type), and from the middle of August to September (Autumn type) (Shin, 1974). They pass the winter in a pupae state and emerge in early April to form the adults of spring type. This specialist herbivore adapted to secondary metabolites of *A. contorta*, particularly aristolochic acids, even it can utilize those metabolites as a defense to its enemies (Nishida, 2002).

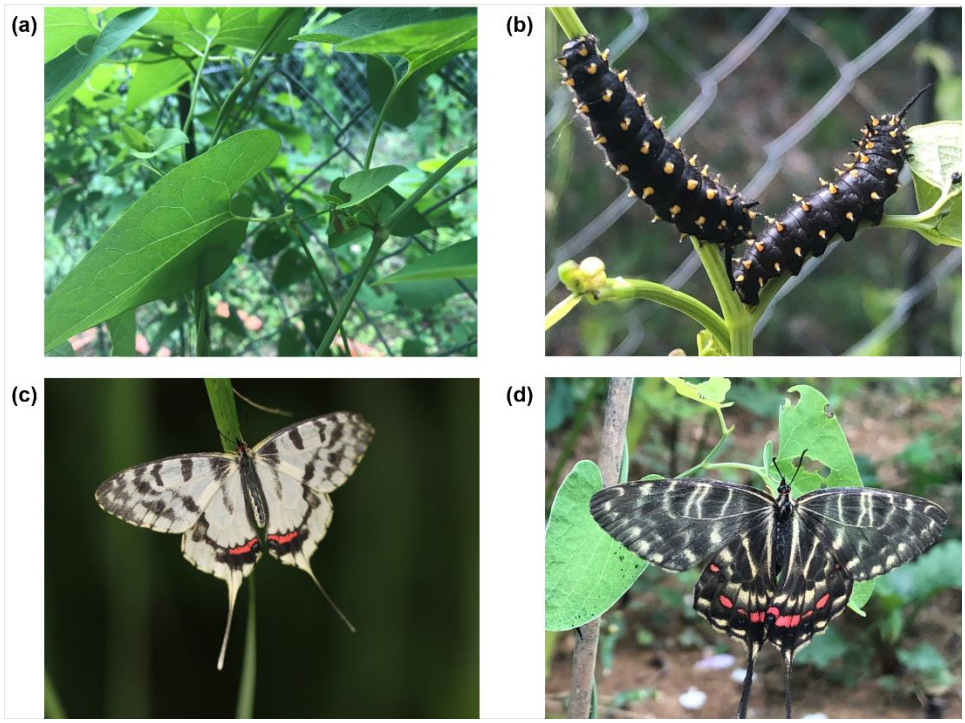


Figure 1-1. Typical appearances of *Aristolochia contorta* Bunge (a), larvae (b), male adult (c), and female adult (d) of *Sericinus montela* Gray, which are experimental species of this research.

Based on the biological and ecological characteristics of *A. contorta*, I thought this plant is appropriate for examination of the possible effect of climate change, especially increased precipitation frequency. In addition, considering the importance of specialist herbivore vulnerable to environmental changes owing to narrow niches (Sax et al., 2013), the effects of climate change on plant-herbivore interaction should be conducted first with specialist herbivore rather than generalist herbivore. In this perspective, the species-specific relationship between *A. contorta* and *S. montela* involving plant secondary metabolites is also thought to be significant reason to use for study about plant and specialist herbivore interaction. Hence, I selected *A. contorta* and *S. montela* as experimental species for studies in the effects of climate change on plant-herbivore interaction.

1.4. Purpose of research

As climate change progresses, it is quite obvious that the future biodiversity would be threatened by rapid environmental changes (Bellard et al., 2012). Thus, the information about detail mechanisms of climate change effect on ecosystem is essential to prevent the decline of biodiversity. Among various populations, considering the value as significant providers of ecosystem services and the large proportion in biodiversity (Basset and Lamarre, 2019), the attempt to understand the effects of climate change on entomofauna should be made. In this context, plant responses to climate change is needed to be examined preferentially because of its significant consequences on insect family (DeLucia et al., 2012). Owing to this importance, many researches have conducted to understand the effects of climate change on various plants, and suggests carbon fertilization effect and nutrient dilution hypothesis as described above (Matthews, 2007; Welti et al., 2020). Nevertheless, since the responses of plant could differ according to species characteristics and climate change progresses in complex ways, the suggestion still requires to be verified thoroughly and also demands more experimental evidences.

Based on these findings, I tried to elucidate how climate change affect the interaction between considerable specialist herbivore and its native host plant, *S. montela* and *A. contorta*, particularly concentrating on cascading effects of plant growth and physiological responses to climate change. Additionally, I attempted to consider seasonal dynamics in plant-herbivore interaction to enable precise and sophisticated understanding. In other words,

this research has three major goals as followings (Fig. 1-2): 1) to examine the possible effects of various environmental factors on ecological traits of *A. contorta*, 2) to address the gap related to individual and/or interactive effects of CO_2 elevations and soil water content on plant-herbivore interaction, which are highly predictable to occur under climate change, considering previous findings of first examination, 3) to fill up the gap related to consideration of seasonal dynamics of plant-herbivore interaction reflecting life cycles of plant and herbivore in nature, and also the effects of climate change on the plant-herbivore interaction considering temporal variations. I expect that the findings of this research could provide detail empirical information about the effects of variable environmental factors under climate change on plant and specialist herbivore interaction, and the possibility of government by species-specific characteristics. Furthermore, this study highlights the importance of increased precipitation frequency as an influential environmental factor in climate change research. Moreover, this study also suggests ecological implications of seasonal dynamics for plausible predictions of future plant-herbivore interaction under climate change.

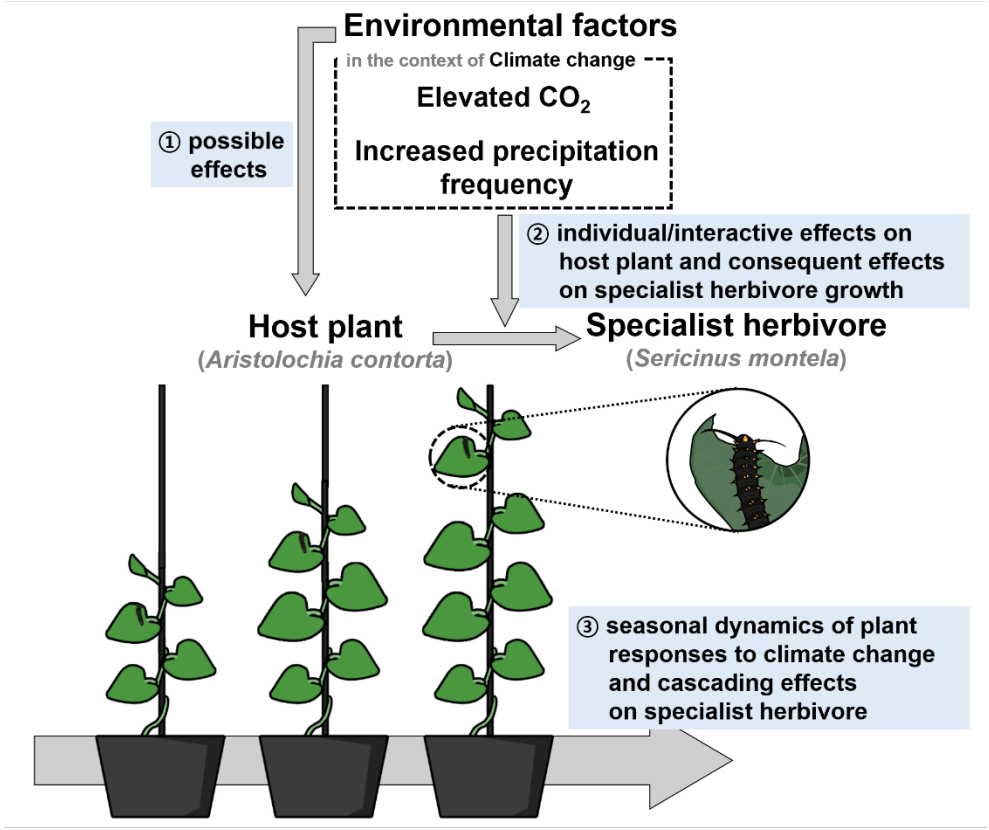


Figure 1-2. A schematic diagram showing purpose of this research. Gray-colored boxes are three major goals of this research.

Chapter 2. Biotic and abiotic effects on the growth and reproduction of *Aristolochia contorta*①

2.1. Introduction

Perennial plants maintain their populations through both of sexual and asexual reproduction. In particular, the effectivity of asexual reproduction in perennial plants could be different according to the growth characteristics such as growth speed and period, since the asexual reproduction is dependent on the amount of nutrient stored during growth period (Cook, 1979). Various environmental factors, i.e. soil properties, climate, competition, herbivore pressure, could affect growth characteristics of plants (Chapin et al., 1987), and those effects of environmental factors are generally species-specific (Klanderud, 2008). Not only growth characteristics but also sexual reproduction properties have close relationships with several environmental factors, which can be shown as changes in number of flowers or timing of flowering (Bichsel et al., 2008; Cho et al., 2016). Particularly, appropriate timing of flowering and fruiting is of importance, as it is closely related to the end of growth period (Bengtsson and Ceplitis, 2000). Therefore, it is necessary to examine the influential environmental factors on growth and reproduction of perennial plants for understanding the maintenance of population and the production of offspring.

Aristolochia contorta Bunge, one of the perennial vine plants, is mostly distributed in East Asia including Korea, Japan, and Russia (Nakonechnaya

① This chapter was published in 'Journal of Wetlands Research 22(3)' in 2020 with minor modifications. (DOI: 10.17663/JWR.2020.22.2.113)

et al., 2012). In Korea, it inhabits waterside region such as riverside and edge of paddy field, generally flowers from July to August, and fruits in October (Lee, 2003). Although the distribution range and population size are not small in Korea, but number of individuals are rapidly decreasing nowadays because of riverside management service and land development project (Choi and Kim, 2011). This decline of *A. contorta* population could be problematic to dragon swallow butterfly (*Sericinus montela* Gray) designated as a vulnerable species (VU) in the red book, since it uses *A. contorta* as an only food plant (Sviridov, 1983). Thus, it is necessary to study habitat characteristics of *A. contorta* and examine the influential environmental factors on growth and reproduction of *A. contorta* for conservation *S. montela* as well as *A. contorta* itself.

Nevertheless, there are few researches about the relationship between *A. contorta* and its habitat environment. Most researchers have been focused on the characteristics of germination (Nakonechnaya et al., 2013; Voronkova et al., 2018), low genetic diversity of population (Nakonechnaya et al., 2012; Nam et al., 2020), and extraction and examination of metabolites in *A. contorta* (Ma et al., 2018; Wen et al., 2006). Recently, a field survey and a mesocosm experiment in context of the relationship between growth of *A. contorta* and the environment were conducted (Park et al., 2019), but it only explained about the effect of support type and light intensity except other environmental factors. Therefore, it is important to study the effect of various environmental factors on growth and reproduction characteristics of *A. contorta* including the field survey of their habitats.

In this study, I tried to study the environmental characteristics of *A.*

contorta habitats and their growth and reproduction properties. In addition, I also tried to figure out the influential environmental factors on growth and reproduction properties of *A. contorta*. I conducted a quadrat survey in four *A. contorta* habitats in South Korea. I examined major accompanying species and herbivore existence as biotic factors, and support type and soil physicochemical characteristics as abiotic factors for potentially effective environmental factors on growth and reproduction of *A. contorta*. My findings could provide fundamental data for understanding the relationship between habitat environment and growth and reproduction properties in *A. contorta*, and also contribute to find essential factors for stable maintenance of *A. contorta* population.

2.2. Methods

2.2.1. Study sites and survey methods

I selected four regions (Gapyeong-gun in Gyeonggi-do, Yeoju-si in Gyeonggi-do, Pyeongtaek-si in Gyeonggi-do, Cheongju-si in Chungcheongbuk-do) among ten regions which are known as *A. contorta* habitats, considering the enough population size and active growth performance (Fig. 2-1). *Aristolochia contorta* population in Gapyeong (GP) was located near the valley, population in Yeoju (YJ), Pyeongtaek (PT), Cheongju (CJ) were situated at the stream side. All of *A. contorta* in four regions were over than 3 years old, which have ability to flower and fruit.

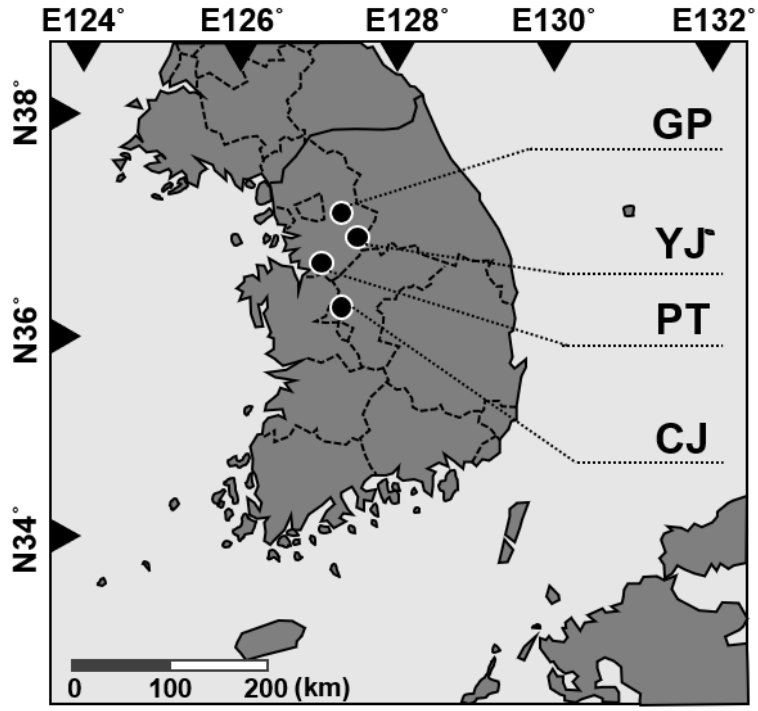


Figure 2-1. Location of surveyed sites. (CJ: Cheongju; GP: Gapyeong; PT: Pyeongtaek; YJ: Yeoju)

Field survey were conducted in July and October 2018, before and after the flowering season. Quadrats of 1 m × 1 m were installed in a regular interval in each region. I measured stem length, number of leaves, total leaf area of *A. contorta*, and checked the flowering and fruiting. I also investigated shoot height, density, and coverage of other accompanying species, and determined major accompanying species for top-three species of important value. In addition, I collected soil samples equally at four vertexes of each quadrat for soil physicochemical analysis. Temperature and precipitation of four regions were compared using data from Korea Meteorological Administration, and there were no significant differences in both of temperature and precipitation throughout all regions (Table 2-1, Korea Meteorological Administration, 2018).

Table 2-1. Average of daily highest and lowest temperature, and monthly total precipitation of each surveyed region (Korea Meteorological Administration, 2018). (CJ: Cheongju; GP: Gapyeong; PT: Pyeongtaek; YJ: Yeoju; SE: Standard Error)

Month	Daily highest temperature (°C) (mean ± SE)				Daily lowest temperature (°C) (mean ± SE)				Monthly total precipitation (mm)			
	CJ	GP	PT	YJ	CJ	GP	PT	YJ	CJ	GP	PT	YJ
Jan.	1.4 ± 0.9	0.9 ± 0.9	0.1 ± 0.9	1.0 ± 0.8	-8.8 ± 1.0	-9.0 ± 0.9	-7.0 ± 0.9	-9.9 ± 1.0	21.5	4.5	5.5	5.0
Feb.	5.0 ± 1.0	3.9 ± 0.8	2.7 ± 0.9	4.1 ± 0.9	-8.8 ± 0.8	-8.1 ± 0.9	-5.4 ± 0.8	-9.2 ± 0.9	29.5	22.0	24.5	29.0
Mar.	14.8 ± 1.1	14.4 ± 0.9	12.8 ± 0.9	14.1 ± 1.0	1.0 ± 0.7	2.0 ± 0.8	3.0 ± 0.7	1.0 ± 0.7	75.5	67.5	68.5	63.0
Apr.	20.3 ± 1.0	20.0 ± 1.0	17.9 ± 0.9	20.0 ± 1.0	5.4 ± 0.5	6.3 ± 0.6	7.5 ± 0.6	5.4 ± 0.6	116.0	141.0	120.0	116.0
May	24.6 ± 0.7	23.8 ± 0.6	22.8 ± 0.6	24.3 ± 0.7	11.8 ± 0.6	11.6 ± 0.6	12.6 ± 0.5	11.4 ± 0.7	94.5	224.5	143.0	226.5
Jun.	28.8 ± 0.5	29.3 ± 0.5	27.4 ± 0.4	29.5 ± 0.5	16.9 ± 0.4	17.1 ± 0.4	17.8 ± 0.3	16.8 ± 0.4	54.5	122.0	97.0	145.0
Jul.	32.6 ± 0.7	32.1 ± 0.8	31.6 ± 0.7	33.1 ± 0.8	22.2 ± 0.4	22.1 ± 0.4	22.7 ± 0.4	22.1 ± 0.4	193.0	246.5	189.0	229.0
Aug.	33.5 ± 0.7	33.0 ± 0.7	32.2 ± 0.8	33.1 ± 0.9	23.1 ± 0.4	22.9 ± 0.5	23.6 ± 0.5	22.4 ± 0.5	256.5	279.0	181.0	379.5
Sep.	25.8 ± 0.4	25.7 ± 0.4	24.8 ± 0.5	25.8 ± 0.5	14.7 ± 0.7	14.6 ± 0.6	16.4 ± 0.5	14.6 ± 0.6	164.5	82.0	84.5	103.5
Oct.	18.8 ± 0.6	18.2 ± 0.6	17.9 ± 0.6	18.1 ± 0.7	6.1 ± 0.7	5.7 ± 0.6	8.0 ± 0.5	5.0 ± 0.6	110.5	116.5	174.5	108.5
Nov.	13.9 ± 0.6	12.9 ± 0.8	12.9 ± 0.6	12.8 ± 0.8	1.1 ± 0.6	1.1 ± 0.7	3.3 ± 0.6	0.1 ± 0.7	39.5	61.5	62.5	53.0
Dec.	5.3 ± 1.0	4.2 ± 0.9	4.0 ± 1.0	4.5 ± 0.9	-6.1 ± 0.9	-6.7 ± 0.9	-4.3 ± 0.9	-7.6 ± 0.9	29.0	17.0	23.0	27.0

2.2.2. Soil analysis

Soil samples were dried in the shade and passed through a 2 mm sieve (standard sieve #10) to remove plant bodies and gravels before analysis. Moisture contents were measured after samples were dried at 105°C in an oven. Organic matter content was analyzed by the loss-on-ignition method (Boyle, 2004) using a 550°C furnace. Soil pH and electric conductivity (EC) were measured from a mixture of 10 g soil samples and 50 ml distilled water using a pH meter (model Starter300, OHAUS) and an electrical conductivity meter (model Starter300c, OHAUS), respectively. NO_3-N and NH_4-N were extracted with 2 M KCl (Merck KGaA, Germany) solution and measured by hydrazine method (Kamphake et al., 1967) and indophenol method (Solorzano, 1969), respectively. PO_4-P was extracted with Bray No. 1 solution, a mixed solution of 1 N NH_4F (Junsei chemical, Japan) solution and 0.5 N HCl (Fisherchemicals, USA) solution (Bray and Kurtz, 1945), and measured by ascorbic acid reduction method (Murphy and Riley, 1962). K^+, Ca^{2+}, Na^+, and Mg^{2+} were extracted with 1 N NH_4CH_3COOH (Kanto Chemical, Japan) solution (Allen et al., 1974) and measured with an atomic absorption spectrometer (model AA240FS, VARIAN).

2.2.3. Statistical analysis

Before statistical analysis, all data were first checked for normality by Shapiro-Wilk test. I conducted *t*-test to compare the growth of *A. contorta* between July and October, and one-way analysis of variance (ANOVA) and Duncan's post-hoc test to compare the growth of *A. contorta* according to regions. These tests were performed at 0.05 significance level. Additionally,

redundancy analysis (RDA), Monte-Carlo permutation test, and principle component analysis (PCA) were conducted for examination of major environmental factors differing four regions. I utilized R (ver. 3.6.1) for all of statistical analysis, particularly package 'agricolae' was used for one-way ANOVA and Duncan's post-hoc test, and package 'devtools', 'vegan' were used for RDA and PCA (R Core Team, 2019).

2.3. Results and discussion

2.3.1. Regional growth characteristics of *A. contorta*

Growth characteristics of *A. contorta* appeared differently according to regions (Fig. 2-2). In July, the longest stem length and largest leaf number was observed in PT (stem length: 228.2 ± 29.3 cm; leaf number: 118.0 ± 17.5), and had fruited among four regions only (Fig. 2-3). Second longest stem length was obtained in GP (188.3 ± 22.1 cm), but leaf number was similar with CJ and YJ (49.5 ± 8.3). In addition, *A. contorta* in GP had flowered but no fruited at all. Contrastingly, *A. contorta* in CJ had the shortest stem length (103.9 ± 12.3 cm), and showed smallest leaf number although the difference with other regions was not significant (32.3 ± 3.6). Unlike stem length and leaf number showing clear differences among regions, single leaf area was the largest in GP (43.3 ± 3.7 cm^2) and similar among other three regions without statistical significance (PT: 36.9 ± 3.4 cm^2; YJ: 34.3 ± 3.1 cm^2; CJ: 30.7 ± 2.0 cm^2).

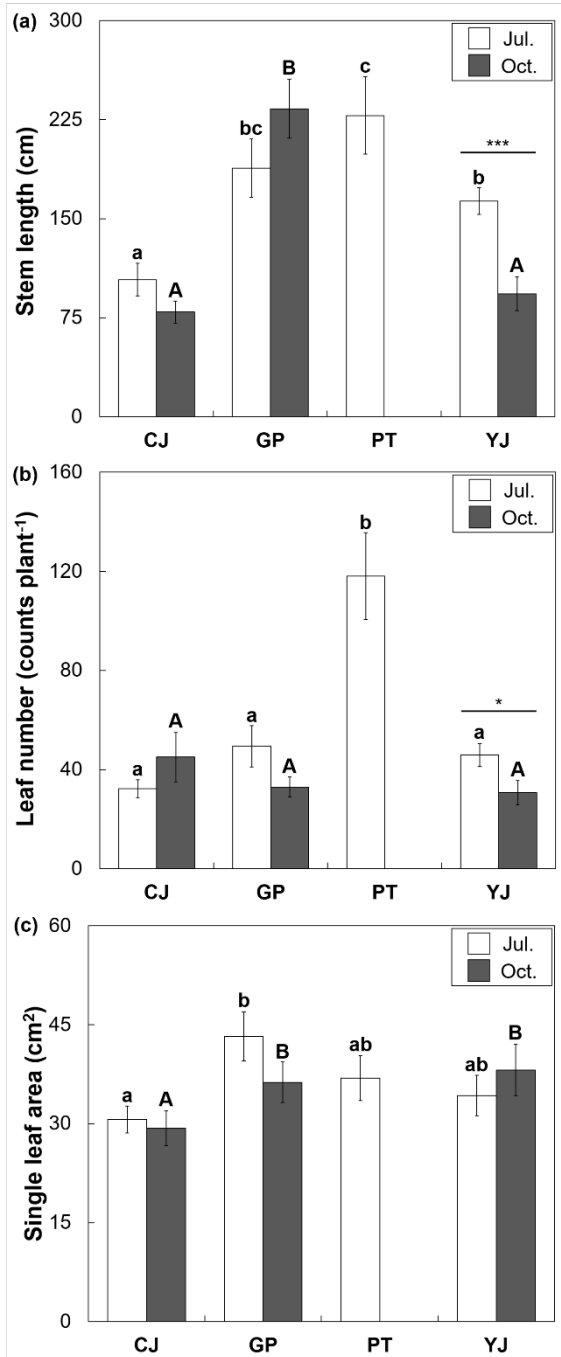


Figure 2-2. Stem length (a), leaf number (b), single leaf area (c) of *A. contorta* for each region. Vertical bars show standard errors. Different letters indicate statistically different sub-groups according to Duncan's post-hoc test ($p < 0.05$). (CJ: Cheongju; GP: Gapyeong; PT: Pyeongtaek; YJ: Yeoju)

Figure 2-3. A fruit of *A. contorta* observed at Pyeongtaek in July

Growth patterns of *A. contorta* in October were distinct from that in July (Fig. 2-2). Most of all, *A. contorta* in PT which showed the most active growth performance in July, all shoots had died as the life cycle ended. In GP, they showed longer stem length compared to July by continuous growth (233.3 ± 22.1 cm), but leaf number reduced slightly (33.0 ± 3.9). This increase and decrease, however, had no any statistical significance. Meanwhile, fruiting was observed in several *A. contorta* including those had flowered in July. In contrast to PT and GP, *A. contorta* in CJ and YJ in October showed shorter stem length, similar or less leaf number than in July, and no flowering and fruiting. Single leaf area of CJ was the lowest (29.3 ± 2.7 cm^2), and that of GP and YJ was similar (GP: 36.3 ± 3.1 cm^2; YJ: 38.1 ± 3.9 cm^2).

Considering these growth characteristics of *A. contorta* before and after flowering season, the most obvious differences among regions were growth speed and timing of flowering and fruiting. In particular, *A. contorta* in PT appeared to show the fastest growth speed, flowering and fruiting timing. The

most vigorous growth, and flowering and fruiting in July was observed in *A. contorta* in PT only (Fig. 2-3). This timing of flowering and fruiting seemed to be faster than the reference which indicates from July to August as flowering season and from September to October as fruiting season (Lee, 2003). In contrast, *A. contorta* in GP showed second largest growth, flowered in July and fruited in October as the reference. In CJ and YJ, no flowering and fruiting has been observed during survey period, and stem length and leaf number decreased after flowering season. This result would be caused by their location where human activities regularly occur. Perennial plants can produce new shoots from rhizome or root bud when their shoots are damaged (Cook, 1979). *A. contorta* could also produce new shoots from root buds if their roots have enough biomass after settlement, as they are also perennial plants. Thus, it is thought that *A. contorta* in CJ and YJ did not showed constant growth nor flowering and fruiting because new shoots had been emerged after July resulted from frequent damage in shoots by anthropogenic management.

2.3.2. Effects of environmental factors on growth speed of *A. contorta*

To examine the effect of environmental factors on growth speed of *A. contorta*, I compared environments of GP and PT where the fastest and second-fastest growth speed appeared. In this study, the fast growth speed of *A. contorta* in PT seemed to be affected by the support type. Supports in PT were artificial constructs or trees, whereas most supports in GP were herbaceous plants or shrubs except some areas where *A. contorta* were

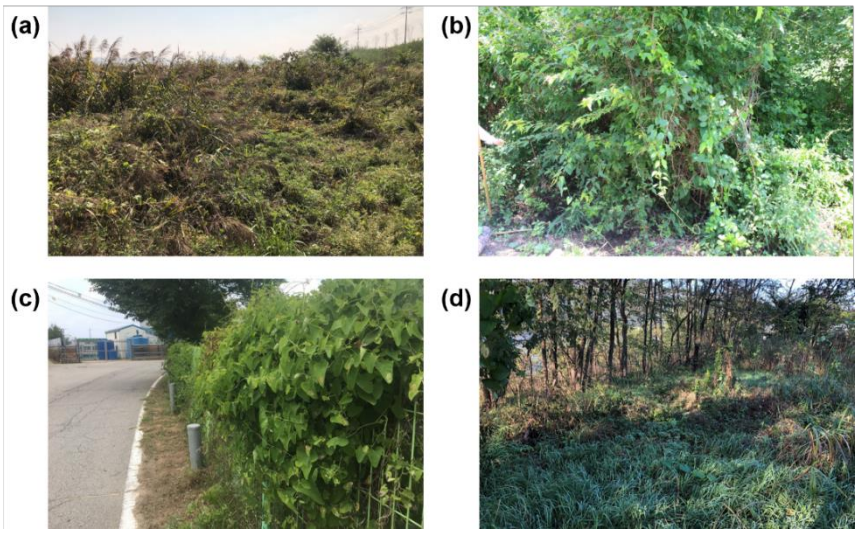


Figure 2-4. The whole view of each surveyed site Cheongju (CJ) (a), Gapyeong (GP) (b), Pyeongtaek (PT) (c), Yeoju (YJ) (d)

relying on trees (Fig. 2-4). Similar with GP, *A. contorta* in CJ and YJ used herbaceous plants or shrubs as their supports. *A. contorta* is able to use any objects as its support, but their stem could elongate longer when their supports are large and stable enough such as artificial constructs or trees (Park et al., 2019). In addition, faster and more active stem growth of vine plants, such as *A. contorta*, could appear when those supports are provided from the early growth period (Gianoli, 2003). Based on these facts, herbaceous plants as supports for *A. contorta* could act as constraints since they cannot grow enough to be stable supports for *A. contorta* in the early growth period. Therefore, *A. contorta* in PT were able to grow fast because they had stable supports from the early growth period, whereas other regions were not.

In addition, I conducted redundancy analysis (RDA) and permutation test to figure out the influential factor among soil characteristics (Fig. 2-5). Among soil factors, soil moisture content seemed to be remarkable for the

growth speed of *A. contorta*. Indeed, the soil moisture content of GP was higher than that of PT in both July and October (Fig. 2-6). High average soil moisture content or frequent water supply could possibly act as a stress to plants adapted to frequent dry soil condition (Keddy, 2017), including A. contorta. Therefore, the growth of *A. contorta* in GP was thought to be delayed by the stress resulted from high soil moisture content.

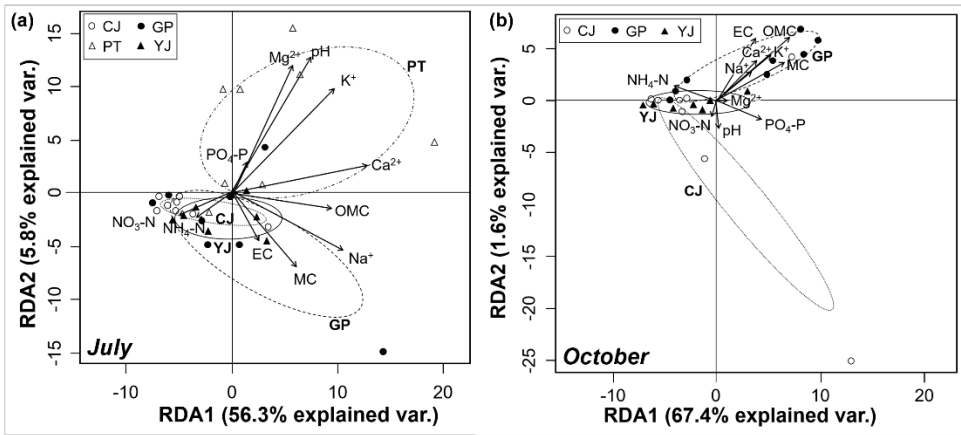


Figure 2-5. Redundancy analysis (RDA) between growth characteristics of A. contorta and soil physicochemical factors for four regions. July (a) and October (b). (CJ: Cheongju; GP: Gapyeong; PT: Pyeongtaek; YJ: Yeoju)

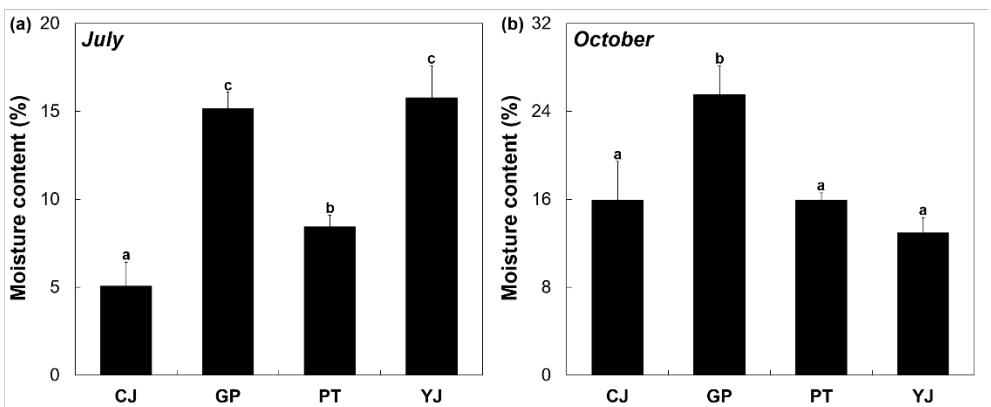


Figure 2-6. Soil moisture content of *A. contorta* habitats in four regions in July (a) and October (b). Vertical bars show standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test ($p < 0.05$). (CJ: Cheongju; GP: Gapyeong; PT: Pyeongtaek; YJ: Yeoju)

2.3.3. Effects of environmental factors on flowering and fruiting of *A. contorta*

To examine the effect of environmental factors on flowering and fruiting of *A. contorta*, I compared environments of GP and PT where flowering and fruiting were checked during whole survey period. In this study, early flowering and fruiting of PT seemed to be influenced by competition stress from accompanying species. Although species composition was different among regions, most of the accompanying species were shrub or herbaceous species with solid and well-developed canopy that can be utilized as supports for *A. contorta* regardless of regions (Table 2-2). In PT, however, *Metaplexis japonica* and *Sicyos angulatus*, which have similar ecological niche with *A. contorta*, were one of the major companion species. Moreover, they showed vigorous growth and development until October with large biomass even when the shoots of *A. contorta* were all died. Thus, intraspecific competition is thought to be higher in PT than other regions because *A. contorta* inhabits with those species of similar ecological niche. This high competition stress might affect fast flowering and fruiting of *A. contorta* in PT, since strong stress from intraspecific competition could change the timing of flowering and fruiting in plants (Weiner, 1988).

Table 2-2. List of companion species of each region. Top three shrub or herbaceous species were showed for each region and month representatively considering the importance value of each species.

Region	Companion species	
	July	October
Cheongju (CJ)	*Robinia pseudoacacia* *Ambrosia trifida* *Phragmites australis*	*Robinia pseudoacacia* *Phragmites australis* *Ambrosia trifida*

Table 2-2. (Continued.)

Region	Companion species	
	July	October
Gapyeong (GP)	*Phragmites australis* *Erigeron annuus* *Artemisia indica*	*Phragmites australis* *Miscanthus sinensis* *Fallopia dumetorum*
Pyeongtaek (PT)	*Metaplexis japonica* *Artemisia indica* *Sicyos angulatus*	*Metaplexis japonica* *Sicyos angulatus* *Artemisia indica*
Yeoju (YJ)	*Miscanthus sacchariflorus* *Phragmites australis* *Artemisia indica*	*Miscanthus sacchariflorus* *Phragmites australis*

Besides companion species with similar ecological niche causing high competition stress, herbivore stress appeared to affect the rapid flowering and fruiting of *A. contorta* in PT. *Byasa alcinous*, one of the specific herbivores on *A. contorta*, was observed in PT only (Fig. 2-7). In fact, few herbivores can use *A. contorta* as a food plant as it contains toxic secondary metabolites including aristolochic acids (Optiz and Müller, 2009). Larvae of

Figure 2-7. A larva of *Byasa alcinous* on *A. contorta* observed at Pyeongtaek in July.

B. alcinous, however, have an ability to overcome those toxic compounds (Nishida and Fukami, 1989), and were found in the density of 14~22 individuals m^{-2} in PT. Since the herbivore stress could advance flowering timing of plant (Brody, 1997), the high herbivore stress in PT seemed to be a reason of early flowering of *A. contorta* in PT.

In addition to biotic factors, cation contents in soil among abiotic factors seemed to be related to early flowering and fruiting of *A. contorta* in PT. According to the result of PCA, soil characteristics of four regions in July were distinct clearly, but those in October were similar except for PT (Fig. 2-8). Among various soil properties, organic matter and cation content such as Ca^{2+} and K^{+} appeared to be significant factors regardless of season, showing high value in PT (Fig. 2-8). Soil cations have close relationships with flowering and fruiting of plants. For instance, excessive inflow of K^{+} in soil can advance flowering timing of plant (Wang, 2007), or increase the number of flowers (Ghosh and Pal, 2010). Additionally, Mg^{2+} plays an important role for successive fruiting of plants (Joham, 1986). Moreover, Ca^{2+} is pivotal for plant signaling pathway including flowering and fruiting (Yang and Poovaiah, 2003), particularly can control the timing of flowering participating in signaling pathway of flowering initiation (Tsai et al., 2007). Therefore, the combined effects of large amount of soil cation and large stress of herbivorous attack are thought to occur the advance in flowering and fruiting of *A. contorta* in PT. In addition, flowering and fruiting are closely connected to the end of growth period (Bengtsson and Ceplitis, 2000), early death of shoots in PT seemed to happen as a consequence of early flowering and fruiting.

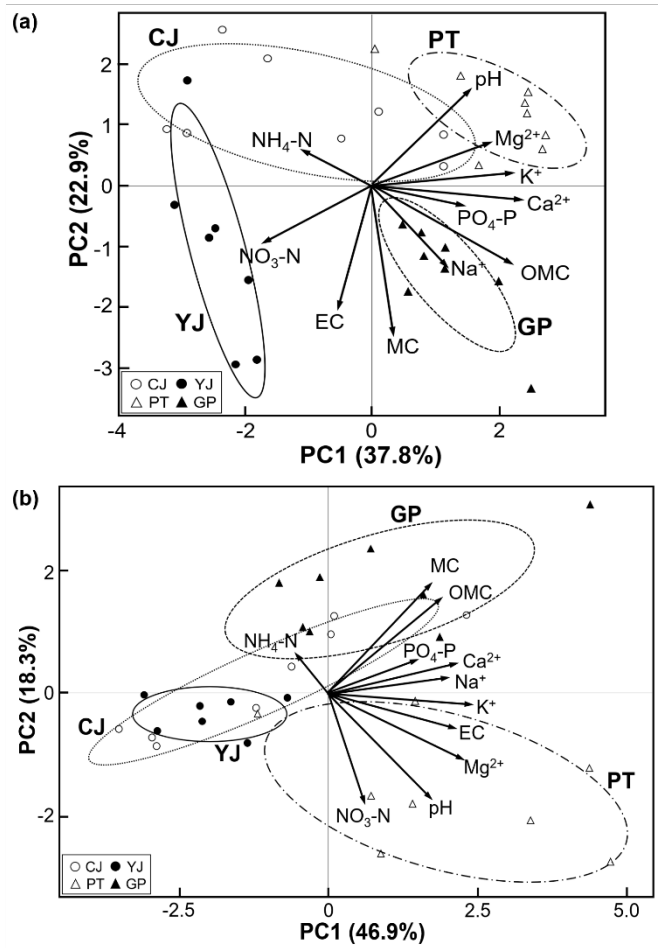


Figure 2-8. Principle component analysis (PCA) of soil physicochemical characteristics for our regions. July (a), October (b). (CJ: Cheongju; GP: Gapyeong; PT: Pyeongtaek; YJ: Yeoju)

2.4. Conclusion

Growth characteristics of four regions in Korea showed differently, and growth speed, flowering timing, and fruiting timing were distinct particularly affected by biotic and abiotic factors. The faster growth speed could be exhibited when the more stable supports such as artificial constructs or trees are provided from the early growth period of *A. contorta*. In addition, the earlier timing of flowering and fruiting would be observed by combined effects of competition and herbivore stress as biotic factors and soil cation contents as abiotic factors unless there is human disturbance. This advance in flowering and fruiting could lead to rapid death of *A. contorta* shoots, and asynchrony in interactions with other insects might occur consequently. Therefore, providing stable supports and alleviating competition and herbivore stress would be necessary for vigorous growth and appropriate timing of flowering and fruiting. Prevention of anthropogenic disturbance which reduces damage in aboveground parts could also be needed for continuous and constant growth of *A. contorta*. Additionally, it would be necessary to verify experimentally the effects of soil moisture content on growth responses of *A. contorta* in detail.

Chapter 3. Reduced host plant growth and increased tyrosine-derived secondary metabolites under climate change and negative consequences on its specialist herbivore[②]

3.1. Introduction

Although the estimates of future diversity vary, there is no doubt that most predictive models indicate alarming consequences for biodiversity under progressing climate change (Bellard et al., 2012). The biodiversity of insects is one of the most considerable issues, as insects are pivotal providers of ecosystem services (Basset and Lamarre, 2019). Indeed, the biodiversity of insects has been dramatically reduced by anthropogenic effects, such as agriculture, pollution, and climate change, and so on (Sánchez-Bayo and Wyckhuys, 2019). Particularly, endangered or vulnerable specialist herbivores, occupying high proportions of insects, are more threatened than generalist herbivores (McKinney, 1997; Piessens et al., 2009; Wilson and Maclean, 2011) since their narrow niches are vulnerable to the rapid effects of climate change (Sax and Bellemare, 2013). Therefore, determining the drivers affecting the specialist herbivore communities under climate change is critical not only for conserving but also for predicting future biodiversity.

As global climate change progress, diverse environmental factors can vary in complex ways (IPCC, 2001). Due to temperature elevations and consequent variations in evapotranspiration patterns caused by elevated

② This chapter was accepted and is in press in 'Science of the Total Environment' in 2020. (DOI: 10.1016/j.scitotenv.2020.143507)

CO_2 concentrations, precipitation patterns under climate change also differ according to its regional characteristics (Dore, 2005; Pfahl et al., 2017). These environmental changes can affect organisms and/or populations directly, and indirectly modify interactions at the community level (Gilman et al., 2010; Walther, 2010). In the case of plant-herbivore interactions, the responses of plants to climate change can have consequent effects not only on plant populations and/or community development (Walther, 2010) but also on their interactions with insects (Delucia et al., 2012). Particularly, the cascading indirect effects of climate change have been considered important mechanisms recently, responsible for the specific interactions (Pires et al., 2020). Thus, it is necessary to examine the responses of plants to environmental changes preferentially for understanding plant-herbivore interactions under climate change conditions.

Among various environmental factors, the effects of elevated CO_2 concentration and temperature on plants have been largely examined in the context of climate change research (Liancourt et al., 2013). For instance, high temperature alters the physiological and/or phenological traits of host plants, causing consequent changes in plant-herbivore interactions (Donoso et al., 2016; Johnson and Hartley, 2017). Additionally, the increased magnitude and/or frequency of precipitation events can individually affect the growth and physiological metabolisms of host plants (Hamerlynck et al., 2000; Mochizuki et al., 2018). Thus, understanding the effects of these environmental factors on plant growth and defenses is necessary to conserve the diversity of entomofauna. In addition, despite the fact that CO_2 elevations and changes in precipitation frequency and intensity occur

simultaneously as a part of climate change, the combinational effects of elevated CO_2 and altered precipitation patterns on plant-insect interactions have not yet received sufficient attention as much as the effects of temperature elevation.

Here, my study addresses this gap by considering how CO_2 elevations and the alteration of soil water content affect the growth and secondary metabolites of a native plant (*Aristolochia contorta* Bunge) and how changes in plants caused by CO_2 elevation and the alteration of soil water content affect the performance of its specialist herbivore (dragon swallowtail, *Sericinus montela* Gray), a species domestically vulnerable to extinction (Fig 3-1). *Aristolochia contorta* is a perennial vine plant, distributed in Eastern Asia including Korea. It usually inhabits forest edges or near riverside (Sviridov, 1983; Korea Forest Service, 2016), showing low range of soil moisture content in their habitats (Park et al., 2019). Therefore, *A. contorta* might be sensitive to increased precipitation frequency or intensity. In addition, *A. contorta* has nitrogen-containing secondary metabolites including aristolochic acids (AAs) (Cheung et al., 2006), and is the only host plant for *Sericinus montela* (National Institute of Biological Resources, 2012). Because of high dependency of *S. montela*, the responses of *A. contorta* to environmental changes would cause remarkable side effects on their interactions.

I hypothesized that host plant growth and defenses would be stimulated under elevated CO_2 concentrations and increased watering frequency based on recent research related to plant photosynthesis and climate change (Je et al., 2018; Lahive et al., 2018). Additionally, I speculated that herbivore

Figure 3-1. Larvae and adults of *Sericinus montela* Gray on *Aristolochia contorta* Bunge

performance might be suppressed according to carbon fertilization by elevated CO_2 concentration (Ainsworth and Rogers, 2007) and increased resistance by enhanced host plant defenses. By measuring plant growth parameters and quantifying plant secondary metabolites, my approach enabled me to determine the host plant responses under elevated CO_2 concentrations and increased watering frequency as well as their interactive impacts. In parallel, I examined the photosynthetic activities to understand the short-term physiological responses against climate change. Finally, I assessed the possible consequences of climate change in specialist herbivorous insects by measuring growth performance as a result of the responses in plants. My findings could contribute to the comprehension of climate change effects on future interactions between plants and endangered insect species.

3.2. Material and Methods

3.2.1. Plant material

The seeds of *Aristolochia contorta* were collected in October 2018 at Pyeongtaek (N 37°05’43”, E 127°05’15”), Gyeonggi-do, South Korea. The seeds were stored in a refrigerator at 4°C under dry conditions. They were sowed in 2019 on mixed soil (sand: topsoil = 2:1, v/v), which considered as similar to the soil texture of natural habitat conditions (Park et al., 2019), and germinated in a greenhouse at Seoul National University, Seoul, Korea. I selected seedlings about 5 cm in shoot height after germination. Each seedling was transplanted into a cylindrical pot (15 cm in diameter, 15 cm in height) filled with a mixed medium of sand (Gang-morae 25 kg, Ecosand, Korea) and topsoil (Superextene, Farm-Hannong, Korea) in a volumetric ratio of 2 to 1 (Table 3-1). This mixture ratio was determined to provide enough nutrients during the experiment considering the soil nutrient condition of natural habitats (Park et al., 2020). A total of 88 seedlings of similar shoot height were transplanted and 22 seedlings were used for each experimental treatment.

Table 3-1. NO_3-N, NH_4-N, and PO_4-P in supplied tap water and experimental soil which is the mixture of sand and topsoil in a volumetric ratio of 2 to 1. Nutrient value of habitat soil was referred to Park et al. (2020).

	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	PO_4-P (mg kg^{-1})
Tap water	0.86 ± 0.01	0.04 ± 0.00	0.01 ± 0.00
Experimental soil	99.34 ± 2.94	54.04 ± 4.72	23.25 ± 1.05
Soil of habitat	1.24 ± 0.12	4.53 ± 1.03	21.88 ± 4.73

3.2.2. Experimental design

The plant growth experiment was conducted from early July 2019 to late October in four hexagonal open-top chambers (OTCs) (1.3m in diameter and 1.1 m in height), referred to Messerli et al. (2015). These OTCs were placed in a greenhouse with open walls to exclude natural rainfall and provide appropriate shade for the plants (about 40% of relative light intensity), referring to Park et al. (2019). I prepared four experimental treatments with two CO_2 concentrations (ambient and elevated) and two watering frequencies (control and increased), and I established each condition in each chamber (Fig. 3-2).

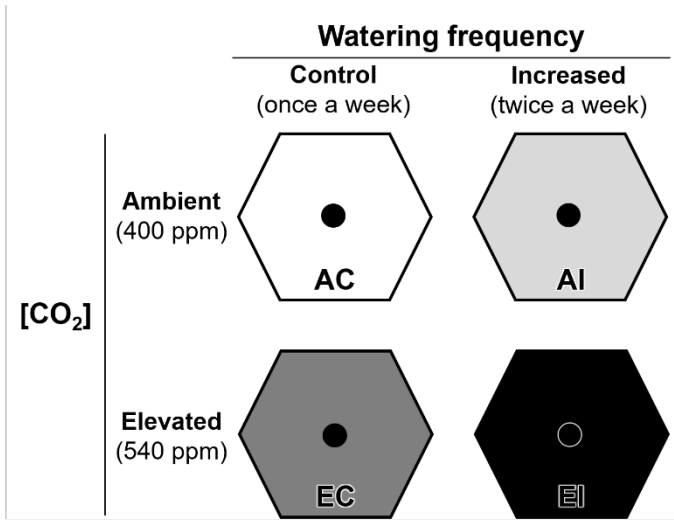


Figure 3-2. A schematic diagram of the experimental design.

The elevated CO_2 concentration of each OTC was controlled with its own CO_2 control system, consisting of a sensor-transmitter coupled with a CO_2 controller (0 – 2,000 ppm_{CO2}, SH-MVG260, Soha-tech, Korea), a solenoid valve, and individual CO_2 gas tanks (99.999%, 40 L) (Messerli et al., 2015) (Fig. 3-3).

Figure 3-3. An established OTC in a greenhouse. A CO_2 controller with an NDIR sensor, a solenoid valve, and a CO_2 gas tank was utilized to control the concentration of CO_2 in the chamber

The target CO_2 concentration of the elevated treatment was 540 ppm, referred to as the RCP4.5 scenario (Thomson et al., 2011). The average CO_2 concentration of the elevated CO_2 chambers was 566.6 ± 4.6 ppm (*n* = 2), and that of the ambient CO_2 chambers was 428.6 ± 3.0 ppm (*n* = 2) (Fig. 3-4). Temperature and relative humidity monitors (HOBO pro v2, Onset, USA) and CO_2 sensors were placed at the center of every chamber, and there were no significant differences in temperature and relative humidity among the chambers (Fig. 3-5). Pots were set around them on a wooden plate. I supplied tap water to all pots in the chambers using a sprinkling can according to the experimental watering frequency (Table 3-1). Water was provided once a week in the control watering frequency environment, while it was provided twice a week in the increased frequency treatment (Fig. 3-6), referring to local precipitation prediction (Cha et al., 2016). When providing water, I made sure to provide enough water (about

600 mL) that the soil of each pot was saturated. Additionally, I monitored soil water content over the entire experimental period to evaluate whether this watering pattern could result in different soil water content patterns, and ensure higher soil water content compared to the natural habitat of the plants in Korea (Fig. 3-6).

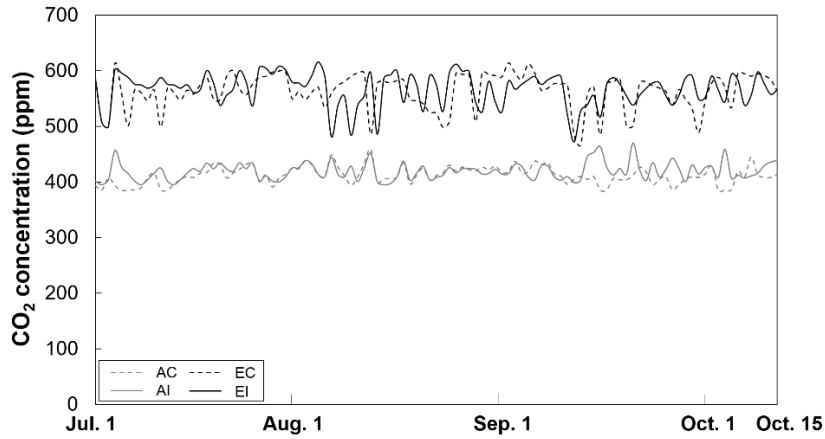


Figure 3-4. CO_2 concentration of each OTC was monitored for a whole experimental period, and the mean value of each CO_2 treatment (elevated and ambient, *n* = 2) of one-day data was shown representatively.

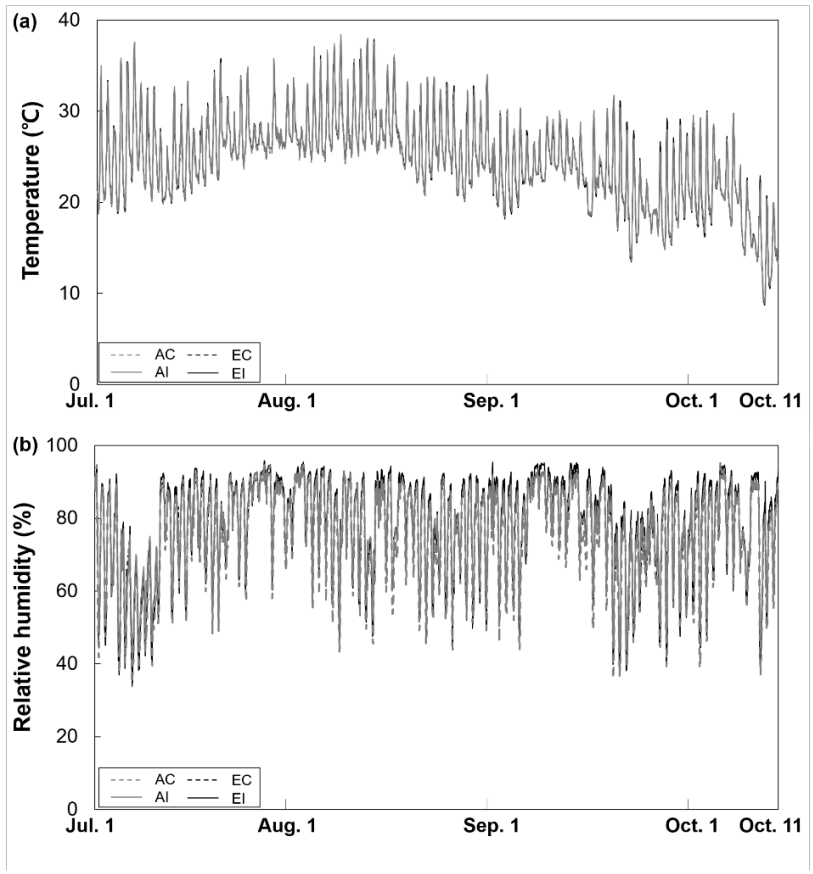


Figure 3-5. Temperature (a) and relative humidity (b) of each OTC were monitored and there were not any significant differences among experimental groups.

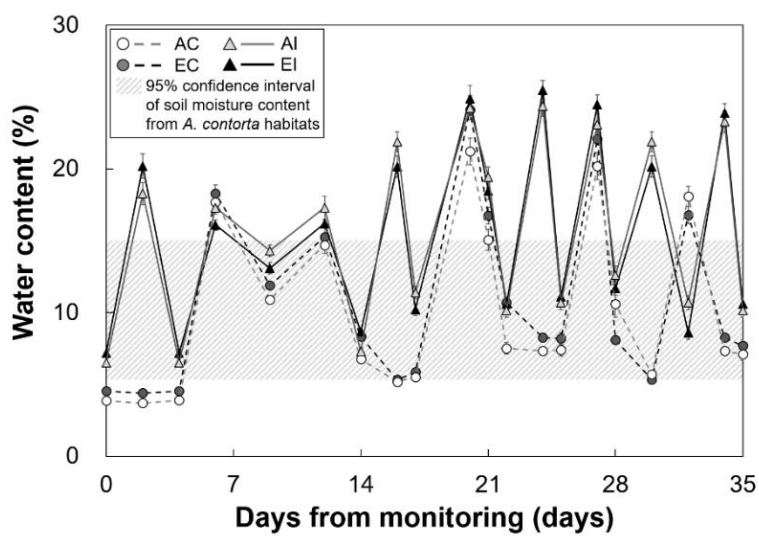


Figure 3-6. Water content variations among the experimental groups, showing the frequency of soil moisture content outside the 95% confidence interval in *A. contorta* habitats

3.2.3. Growth measurement

Stem length and leaf number were measured once a week in 15 representative plants of each condition in 2019. All of the aboveground parts were harvested at the end of the experiment, and divided into stems and leaves. The total leaf area for each plant was measured with LI-3000C and LI-3050C transparent belt conveyers (LI-COR, USA). After the leaf area measurements, the dry weight of the aboveground biomass was determined by drying the stems and leaves in a dry oven at 60°C over three days at the end of the experiment. To validate the growth pattern of *A. contorta* in 2019, I measured stem length and leaf number of *A. contorta* in August 2020, which were grown in the same experimental design.

3.2.4. Carbon and nitrogen analysis

To examine the leaf C: N ratio as a measure of food quality, I conducted stoichiometric analyses of the total carbon (TC) and total nitrogen (TN) ratio in the leaves of the plants. The plant leaves were dried in a dry oven at 60°C and ground (Wiley Mini-Mill 3380L10, Thomas, USA) to make homogeneous mixtures for the stoichiometric analysis. The TC and TN contents were measured using an elemental analyzer (Flash EA 1112, Thermo Electron, USA) at the National Instrumentation Center for Environmental Management (NICEM) at Seoul National University. I calculated the TN in the plants based on the total leaf dry weight and the TN ratio, which is related to nitrogen availability.

3.2.5. Secondary metabolite extraction and instrumental UPLC conditions

To analyze the secondary metabolites, I collected from the fifth to the seventh leaf from the top as samples. Leaf sample collection was conducted twice, in the middle (August) and the end (October) of the experimental period. In August, the samples were collected from seven representative plants for each condition, which were not included in the growth measurements. In October, the samples were collected from 15 plants immediately after harvesting. After collection, I immediately froze the leaves in liquid nitrogen and stored them at -80°C. The leaf samples were ground in a frozen state, and the metabolites were extracted from 50 mg of the ground sample with 1 mL of 40% methanol (HPLC grade, Sigma-Aldrich, USA) containing 0.1 $\mu g \cdot mL^{-1}$ of tribenzylamine (TBA) (≥ 99.0%, Sigma) as an internal standard.

The secondary metabolites were measured in the extraction using an ultra-performance liquid chromatography (UPLC)-quadrupole orthogonal time of flight mass spectrometer (qTOFMS) (Waters ACQUITY UPLC, Micromass Q-Tof micro, Waters, USA) as described by Mao et al. (2017) with modifications. A UHPLC C18 column (2.1 mm × 100 mm, I.D., 1.7 μm, ACQUITY UHPLC® HSS, Waters, USA) was used for the analysis coupling with a C18 pre-column (2.1 mm × 5 mm, I.D., 1.7 μm, Vanguard TM HSS, Waters) and the column were maintained at 40°C. Mobile phase consisting of distilled water with 0.2% formic acid (A) and acetonitrile with 0.2% formic acid (B) was pumped at a flow rate of 0.4 $ml \cdot min^{-1}$. Gradient elution program was as follows: 0 min, 10% B; 2 min, 50% B; 7 min, 95% B; 11 min, 95% B,

15 min, 45%. The injection volume were 2 μL. Eluted compounds were detected from m/z 100 to 1000 using Xevo G2-XS QTOF mass spectrometer (Waters, Manchester, UK) which was connected to an electrospray ionization (ESI) source interface with positive mode using the following instrument setting: drying gas (N_2) flow rate, 8 $mL \cdot min^{-1}$; drying gas temperature, 300ºC; nebulizer pressure, 45 psi; capillary voltage, 3500 V; nozzle voltage, 500 V; fragmentor, 120 V; fixed collision energies were 15 and 30 eV respectively. Data acquisition was achieved using Masslynx v 4.1 (Waters, Milford, MA, USA).

Among the secondary metabolites, the exact concentration of aristolochic acids 1 and 2, well-known secondary metabolites of *A. contorta*, were determined using reaction curves (Appendix 2). Aristolochic acid 1 (AA1) and 2 (AA2) were purchased from Sigma- Aldrich, USA.

3.2.6. Photosynthesis and chlorophyll measurement

Since the leaves of *A. contorta* under elevated CO_2 concentrations were too small to measure the photosynthesis ability, I established new groups of plants in each treatment to examine the effect of climate changes on photosynthesis in *A. contorta*. Twelve plants germinated simultaneously with the experimental plants, but grown outside of the chambers, with similar stem length and number of leaves were selected, and their photosynthetic performances were measured with an LI-6400XT (LI-COR, USA). Considering the V_{cmax} and J_{max} calculated from this measurement, I shuffled and divided the twelve plants into four statistically similar groups (Fig. 3-7), and inserted them into each OTC in late August. These newly

placed plants were grown under each treatment condition for a month, and the photosynthetic performances were measured again in late September. The leaf chlorophyll content of each plant was monitored using a chlorophyll meter (SPAD-502plus, Konica Minolta, Japan), and the relative decrease in production rate was calculated as the ratio of decreased chlorophyll content to the initial chlorophyll content of each plant.

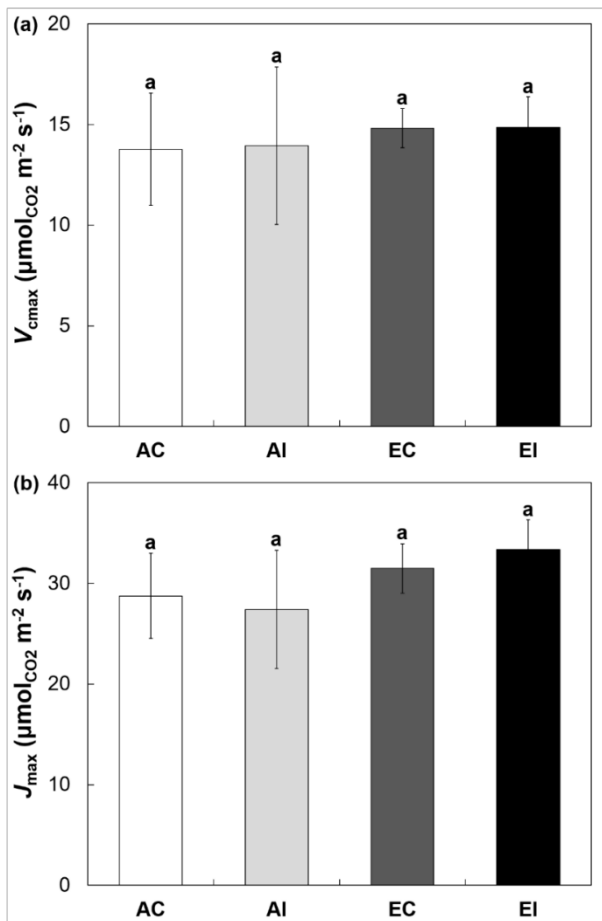


Figure 3-7. Photosynthesis capacity of all experimental groups was not different before treatment. V_{cmax} (a), and J_{max} (b) of before exposure to experimental conditions for each group (n = 3 for each group). Vertical bars show standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test (p < 0.05). (AC: ambient CO_2 concentration and control watering frequency; AI: ambient CO_2 concentration and increased watering frequency; EC: elevated CO_2 concentration and control watering frequency; EI: elevated CO_2 concentration and increased watering frequency)

3.2.7. Measurement of relative growth performance of *Sericinus montela*

The eggs of *S. montela* were collected on September 11, 2019, and they hatched after two or three days. They were raised for three days more with sufficient *A. contorta* leaves and space to ensure that all larvae were in the second instar stage. On September 16th, the leaves were collected from plants not included to growth measurement in each chamber. The petiole of each leaf was immersed in a 1.5 mL tube (Eppendorf, Germany) and sealed with parafilm (Bemis, USA) to prevent drying. Aluminum foil was used to make supports for the tubes, enabling the larvae on the leaves to move freely (Fig. 3-8). A total of 120 larvae were selected based on weight, and 30 larvae were used for each experimental treatment. Before leaf insertion, I took a photo of all leaves of each treatment to calculate the leaf area consumed.

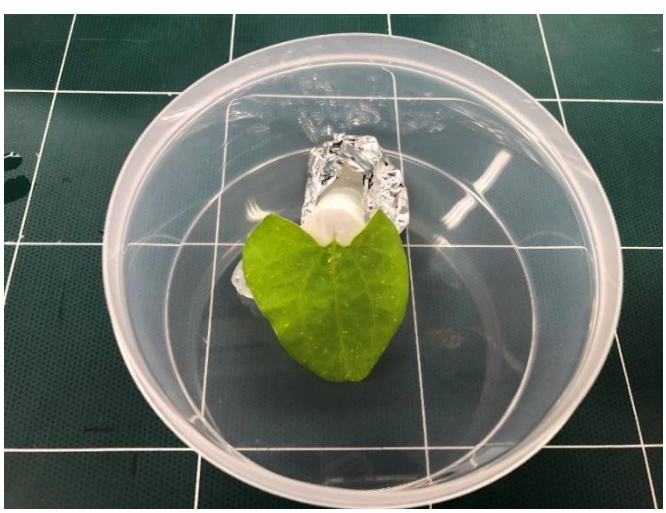


Figure 3-8. An installed tool for bioassay of *S. montela* consists of 1.5 mL tube, aluminum foils, and a leaf of *A. contorta*. This tool was developed to prevent leaf dehydration, leaf adjoining to the bottom of petri dish, and to make a leaf hanged like a real plant to enable the larvae on the leaves move freely.

Additional leaves were inserted on September 19th, when most of the leaves were consumed over 50%. The final weights of the larvae were measured on September 22nd. This bioassay was performed for six days, and the relative growth rate (RGR) was calculated as the ratio of the amount of increased weight to the initial weight of each larva.

3.2.8. Statistical analysis

Two-way analysis of variance (ANOVA) of CO_2 concentration and watering frequency was conducted after checking the normality of the data by the Shapiro-Wilk test. Statistical differences between the experimental conditions were examined by Duncan's post-hoc test. The tests were performed at the 0.05 significance level using R version 3.6.1 (R Core Team, 2019). In addition, the Gompertz model for stem length and leaf number was conducted using R version 3.6.1 with the 'fit' function (R Core Team, 2019), according to the following equation (Zwietering et al., 1990):

$$y(t) = M\, e^{-e^{\{\frac{\mu e}{M}(\lambda - t)+1\}}} \quad \text{(Eq. 1)}$$

where y(t) is the stem length or leaf number in week t (weeks from the start of the experiment) of *A. contorta* under each treatment. Constants M, μ, and λ represent maximum values (cm or number), maximum slope (cm $week^{-1}$ or counts $week^{-1}$), and lag time, the time when the value is 6.6% of M, respectively.

3.3. Results

3.3.1. Differences in plant growth between the experimental groups varied according to organs

The growth performance of *A. contorta* differed between the experimental groups, but the differences were relatively dissimilar according to plant organs (Fig. 3-9). The highest aboveground dry weight (AGDW) was obtained under ambient CO_2 concentrations and control watering frequency conditions. Although the AGDW of *A. contorta* under the AI treatment was slightly higher compared to the elevated CO_2 concentration groups (EC and EI), the differences were not statistically significant (Fig. 3-9a). This pattern was significantly related to both the individual and combined effect of CO_2 concentration and watering frequency (Table 3-2). The leaf dry weights showed patterns similar to the total AGDW (Fig. 3-9b). Under ambient CO_2 concentrations, higher leaf dry weight was observed compared to those in elevated CO_2 concentration conditions and also differed according to the watering frequency. In contrast, under elevated CO_2 concentrations, despite the fact that the lowest leaf dry weight was seen in control watering frequency, there was no significant difference between the increased watering frequency group. Additionally, like AGDW, there were both individual and interactive effects of CO_2 concentration and watering frequency on leaf dry weight (Table 3-2). Stem dry weight, however, showed a different pattern than leaf dry weight and AGDW, with a clear difference only when both CO_2 concentration and watering frequency changed (Fig. 3-9c). The stem dry weight of *A. contorta*

under AC was the highest. Stem dry weight was reduced under elevated CO_2 conditions, and the decrease was even larger when the watering frequency was increased. Belowground dry weight (BGDW) showed the similar pattern with leaf dry weight and AGDW (Fig. 3-9d).

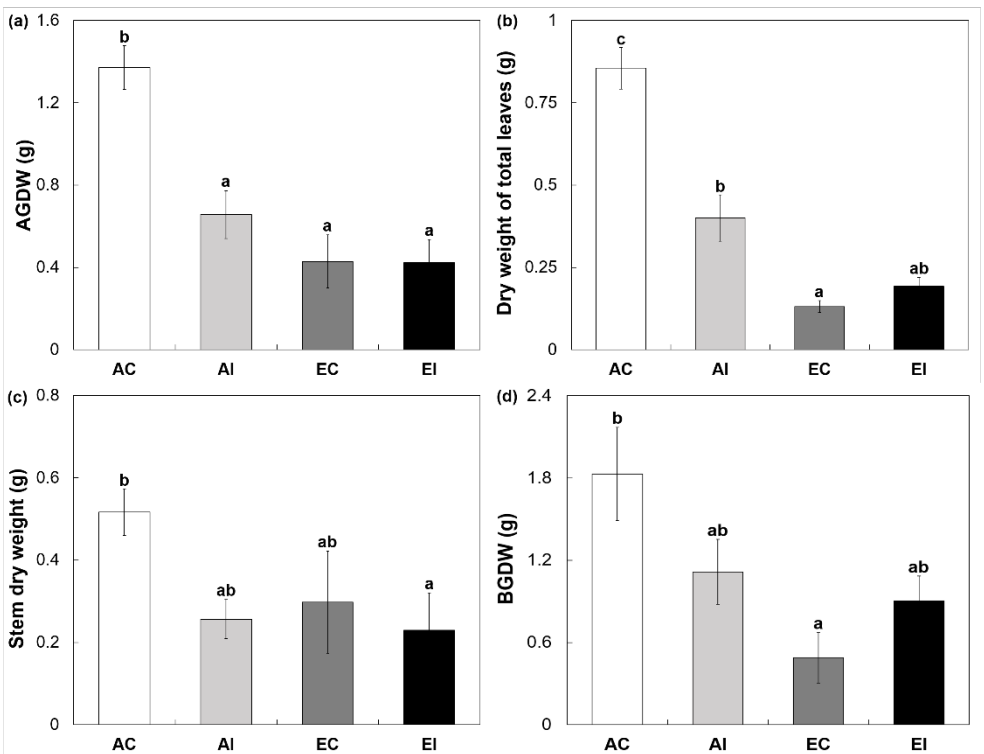


Figure 3-9. Biomass production was interrupted by both elevated CO_2 concentration and increased watering frequency, particularly in the leaves. The total aboveground dry weight (AGDW) (a), dry weight of total leaves (b), stem dry weight (c), and belowground dry weight (BGDW) (d) for each experimental group (n = 15 for AGDW, dry weight of total leaves, and stem dry weight, n = 7 for BGDW). The vertical bars show the standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test ($p < 0.05$).

Table 3-2. *F*-values and *p*-values from two-way analysis of variance (ANOVA) results of the effects of CO_2 concentration and watering frequency on growth, photosynthesis, secondary metabolites of *A. contorta*, and herbivore performance of *S. montela* in an OTC experiment. Degrees of freedom (*df*) of all experimental treatments were 1. Maximum value (M) and maximum slope (μ) are constants from fitted Gompertz models. Statistically significant effects are represented in boldface.

			CO_2		Watering frequency		CO_2 × Watering frequency	
			F	*p*	*F*	*p*	*F*	*p*
Growth		Maximum value (stem length) (cm)	**58.06**	**0.000**	0.923	0.341	**16.26**	**0.000**
		Maximum slope (stem length) (cm $week^{-1}$)	**31.73**	**0.000**	0.685	0.412	**4.057**	**0.049**
		Maximum value (leaf number) (counts)	0.287	0.595	**55.61**	**0.000**	0.336	0.564
		Maximum slope (leaf number) (counts $week^{-1}$)	2.429	0.125	**22.07**	**0.000**	0.653	0.423
		Stem length (cm) (2020)	**78.30**	**0.000**	**4.107**	**0.046**	1.022	0.315
		Leaf number (counts $plant^{-1}$) (2020)	**6.314**	**0.014**	**41.91**	**0.000**	2.098	0.152
		Dry weight of stem (g)	2.065	0.156	3.690	0.060	1.265	0.266
		Dry weight of total leaves (g)	**86.49**	**0.000**	**15.43**	**0.000**	**26.62**	**0.000**
		Total aboveground dry weight (g)	**25.61**	**0.000**	**9.626**	**0.003**	**9.291**	**0.004**
		Total belowground dry weight (g)	**10.04**	**0.008**	0.381	0.549	**5.295**	**0.040**
Photosynthesis		Relative decrease rate of chlorophyll content	**62.49**	**0.000**	0.181	0.682	**14.24**	**0.005**
		V_{cmax} (after treatment) (μmol_{CO2} m^{-2} s^{-1})	**7.084**	**0.029**	0.485	0.506	1.432	0.266
		J_{max} (after treatment) (μmol_{CO2} m^{-2} s^{-1})	1.648	0.235	0.036	0.854	0.238	0.639
C: N ratio		C: N ratio (August)	4.020	0.061	**21.38**	**0.000**	0.18	0.676
		C: N ratio (October)	**83.34**	**0.000**	**77.09**	**0.000**	1.36	0.248
Secondary metabolite contents	August	Magnocurarine (peak intensity)	**29.15**	**0.000**	1.635	0.214	1.167	0.292
		Magnoflorine (peak intensity)	**16.99**	**0.000**	0.016	0.900	0.004	0.951
		Aristolochic acid 1 (ng mg^{-1})	2.905	0.102	0.156	0.696	0.156	0.697
		Aristolochic acid 2 (ng mg^{-1})	1.319	0.263	0.389	0.539	0.330	0.572
	October	Magnocurarine (peak intensity)	**76.59**	**0.000**	**14.06**	**0.000**	0.178	0.674
		Magnoflorine (peak intensity)	**31.59**	**0.000**	**6.997**	**0.010**	**4.250**	**0.043**
		Aristolochic acid 1 (ng mg^{-1})	**8.842**	**0.004**	**5.814**	**0.019**	**4.843**	**0.031**
		Aristolochic acid 2 (ng mg^{-1})	1.395	0.242	1.203	0.277	1.211	0.275
Herbivore performance		Relative growth rate of *S. montela* (day^{-1})	**14.47**	**0.000**	**10.25**	**0.002**	0.113	0.738

Besides the final aboveground biomass production, elevated CO_2 concentration and increased watering frequency affected the patterns of stem elongation and leaf increments differently (Fig. 3-10). The stem elongation pattern differed according to the experimental conditions, especially the CO_2 concentration (Fig. 3-10a, Table 3-2). The highest and second-highest stem length values were obtained under ambient CO_2 concentrations during the entire experimental period, whereas the lowest value was seen in the EC group. *A. contorta* under EI conditions showed higher stem length values than the EC group, but the difference was not significant. To examine the differences in the patterns in detail, I further fitted the stem length growth of the plants into the Gompertz model and compared the maximum slope (μ), which represents the maximum growth speed (Zwietering et al., 1990). Like the maximum stem length value, the maximum growth speed (μ) was significantly affected by the CO_2 level, not by the watering frequency (Table 3-2), showing higher values under ambient CO_2 concentrations (Fig. 3-10a). Although the AC group showed higher maximum stem lengths than the AI group, these two groups showed the same maximum growth speed (Fig. 3-10a). In contrast to the stem length, the leaf increment pattern was distinctly different with watering frequency only (Fig. 3-10b, Table 3-2). The leaf number was lower under increased watering frequency conditions than in the control condition, regardless of the CO_2 concentration. I also fitted the leaf number increments of the plants into the Gompertz model to compare the maximum increasing speed (μ). The maximum increasing speed was affected by the watering frequency only. However, the effect among the experimental

groups was less significant than the maximum growth (Fig. 3-10b, Table 3-2). The AI, EC, and EI groups had differences in maximum growth, but there was no significant difference in maximum speed (Fig. 3-10b). Additionally, the total leaf area per plant, which is an important proxy to estimate the amount of available food to specialist herbivores, showed a pattern similar to leaf dry weight and stem length (Fig. 3-11).

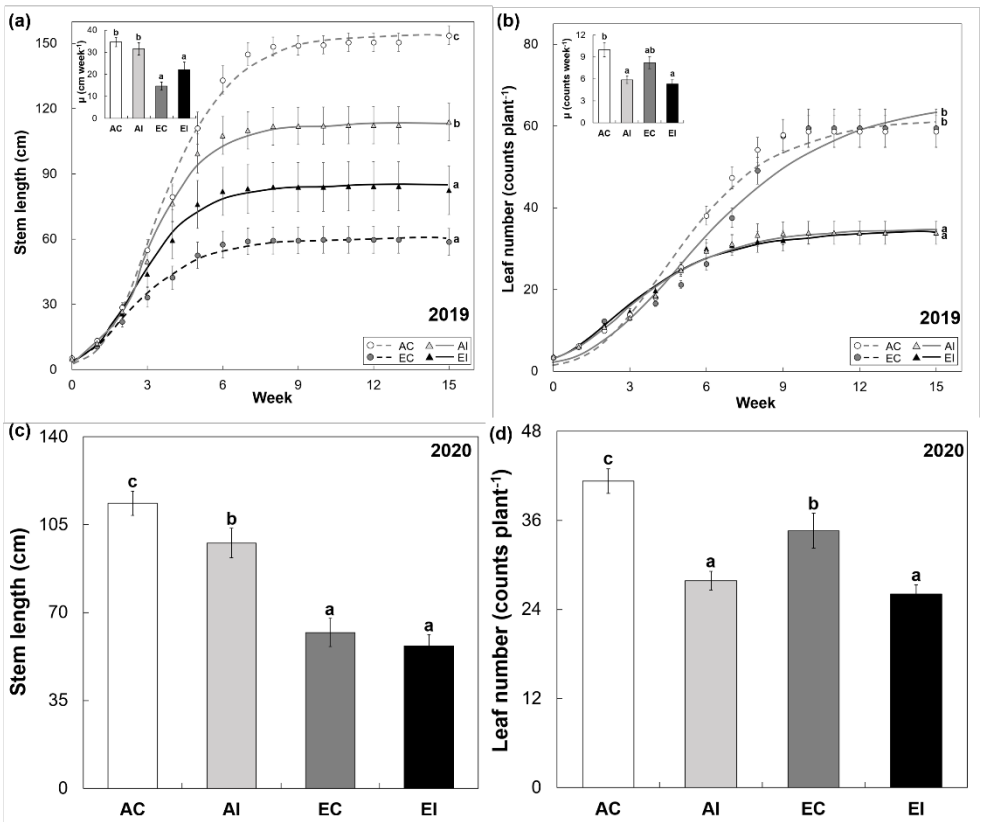


Figure 3-10. Effects of elevated CO_2 concentration and increased watering frequency varied according to plant organs and growth stages, and the same result was obtained in 2020. Stem length (a) and leaf number (b) according to CO_2 concentration and watering frequency during the experimental period in 2019 (*n* = 15 for each condition), Stem length (c) and leaf number (d) for each experimental group in August 2020 (*n* = 20 for each condition). The dots (circle and triangle) show the mean of stem length and leaf number value for each week, and the lines represent the Gompertz model fitted for each condition. The vertical bars show the standard error for each group each week. Different letters indicate statistically different sub-groups by Duncan's post-hoc test (*p* < 0.05).

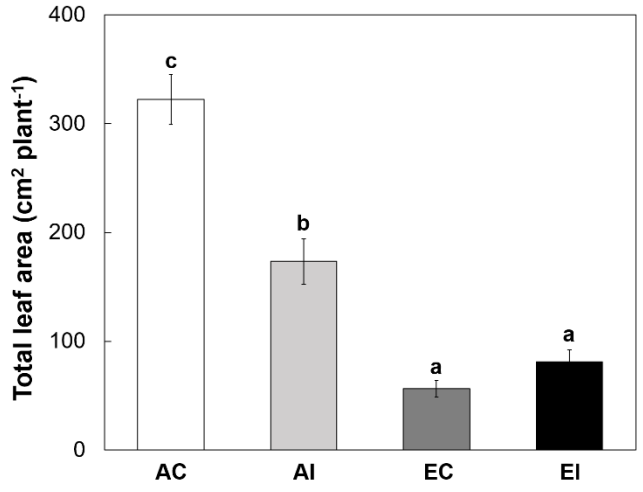


Figure 3-11. Both elevated CO_2 concentration and increased watering frequency reduced total leaf area of plants, and the effect of CO_2 was much more significant. A total leaf area per plant for each experimental group (*n* = 15 for each group). Vertical bars show standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test (*p* < 0.05).

Consistently, stem length and leaf number in August 2020 showed the same patterns with 2019 (Fig. 3-10c, d). Stem length affected by CO_2 concentration more strongly (Table 3-2), and the largest and second largest value was obtained in AC and AI, respectively (Fig. 3-10c). The value of stem length was smaller under elevated CO_2 concentration, and there was no significance between EC and EI (Fig. 3-10c). In contrast, leaf number affected by watering frequency more strongly (Table 3-2), showing larger values under control watering frequency condition than under increased watering frequency condition (Fig. 3-10d).

3.3.2. Photosynthesis inhibition by a month exposure to elevated CO_2 concentration in *Aristolochia contorta*

To understand the physiological responses under the experimental conditions, I further examined changes in the chlorophyll content and photosynthetic performance (Fig. 3-12). The relative decreases in the rates of chlorophyll contents and the ratio of the decreased chlorophyll content to the initial chlorophyll content, were higher under an elevated CO_2 concentration than those under ambient CO_2 concentration (Fig. 3-12a). The largest decreased rate was obtained under EC conditions, whereas the smallest value was observed under AC conditions.

The kinetics of Rubisco (V_{cmax}) and the maximum rate of carboxylation limited by electron transport (J_{max}) were calculated by the relationship between the carbon assimilation rate (A) and the internal CO_2 concentration of a leaf (C_i) and showed different patterns according to the experimental conditions. Before treatment, the V_{cmax} and J_{max} for all experimental groups were similar (Fig. 3-7). However, after treatment, V_{cmax}, which is related to the Rubisco capacity, was lower under elevated CO_2 conditions than under ambient CO_2 concentrations (Fig. 3-12b), indicating a significant effect of CO_2 concentration (Table 3-2). In particular, *A. contorta* under EC conditions showed the lowest V_{cmax}. Although the V_{cmax} of *A. contorta* was slightly higher when the watering frequency was increased under an elevated CO_2 concentration (EI), the difference was not statistically significant. Under ambient CO_2 concentrations, the highest V_{cmax} was obtained with control watering frequency (AC), but the two ambient CO_2 concentration groups (AC and AI) were classified into the same subgroups

based on post-hoc tests. In contrast to the V_{cmax} pattern, there were no significant differences in J_{max} between the experimental groups (Fig. 3-12c, Table 3-2).

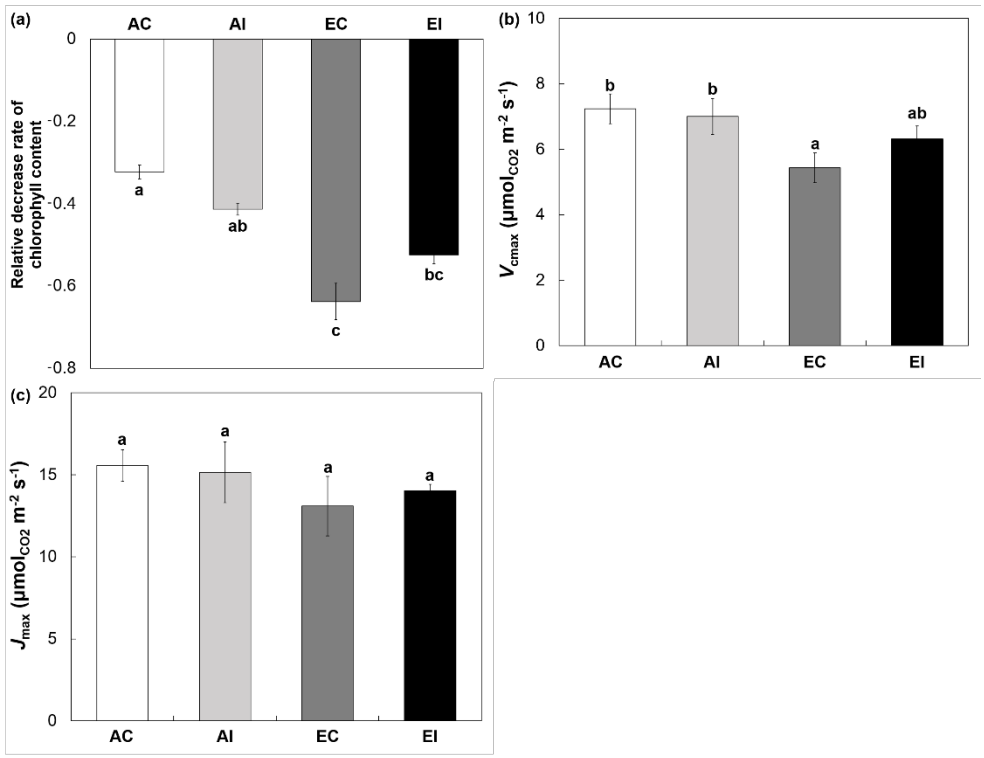


Figure 3-12. Elevated CO_2 concentrations led to decreases in chlorophyll content and Rubisco activity, with slight compensation by increased watering frequency. Relative decrease rate of chlorophyll content (a), V_{cmax} (b), and J_{max} (c) for each experimental group (n = 3 for each group). The vertical bars show the standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test (p < 0.05).

3.3.3. Reduced growth performance of a specialist herbivore caused by decreased food quality of the host plant under climate change

The relative growth rate (RGR) and total leaf area consumed by *S. montela*, a species-specific herbivore of *A. contorta*, were measured to determine the consequent effects of climate change on herbivores (Fig. 3-13). The patterns of RGR and the leaf area consumed were similar. The highest RGR was obtained under AI treatment, whereas the lowest RGR was observed under EC treatment. The RGR of the other two groups, EI and AC, showed intermediate RGR levels (Fig. 3-13a). This pattern was clearly related to the individual variables of CO_2 concentration and watering frequency, but there was no interactive effect (Table 3-2). Like RGR, the leaf area consumed by the herbivore was the highest under AI conditions and the lowest under EC conditions (Fig. 3-13b).

The carbon: nitrogen (C: N) ratio was examined to verify the nutrient dilution hypothesis considered in this study, and secondary metabolites were analyzed as parameters of food quality, responsible for the feeding and growth performance of the herbivore (Fig. 3-14). Prior to analysis of the C: N ratio and secondary metabolite content, I checked for nitrogen limitations by determining the total nitrogen amount per plant, calculated by multiplying the total leaf dry weight and TN ratio for each plant (Fig. 3-15). The largest amount was obtained under AC treatment, where the growth performance was the highest, and the other groups showed statistically similar values. The C: N ratio was decreased in October compared to in

August, and varied according to both the CO_2 concentration and the watering frequency (Fig. 3-14a). Regardless of the season, the C: N ratio of *A. contorta* was decreased under elevated CO_2 concentrations, but increased by watering frequency (Table 3-2).

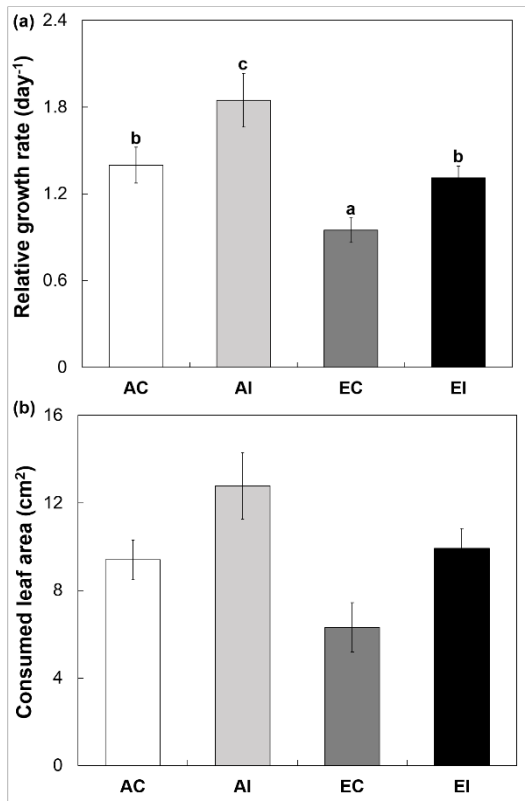


Figure 3-13. Negative impacts of elevated CO_2 concentrations on herbivore growth performance were observed but were partially ameliorated by increased watering frequency. Relative growth rate (RGR) of *S. montela* (a), consumed leaf area during the bioassay experiment (b) for each experimental condition (*n* = 30 for each group in both RGR and leaf area consumed). The vertical bars show the standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test (*p* < 0.05).

I further measured the plant secondary metabolites in *A. contorta* leaves (Fig. 3-14b-e, Fig. 3-16). In August, the magnocurarine, magnoflorine, aristolochic acid 1 (AA1), and aristolochic acid 2 (AA2) content was lower under ambient CO_2 conditions compared to elevated CO_2 conditions. However, no differences according to watering frequency were observed under both CO_2 concentrations (Fig. 3-14b-e, Table 3-2). The patterns of secondary metabolite contents were different in October. First of all, the overall content of secondary metabolites increased compared to August, except for the AA1 and AA2 content (Fig. 3-14b-e). Like in August, the content of magnocurarine, magnoflorine, AA1, and AA2 were higher in elevated CO_2 conditions than in ambient CO_2 concentrations. In contrast, unlike in August, the differences according to watering frequency were clearer in October, particularly in magnocurarine and magnoflorine (Fig. 3-14b, c, Table 3-2). For all four metabolites, the content was less under increased watering frequency treatment than in the control watering frequency treatment, regardless of CO_2 concentration (Fig. 3-14b-e).

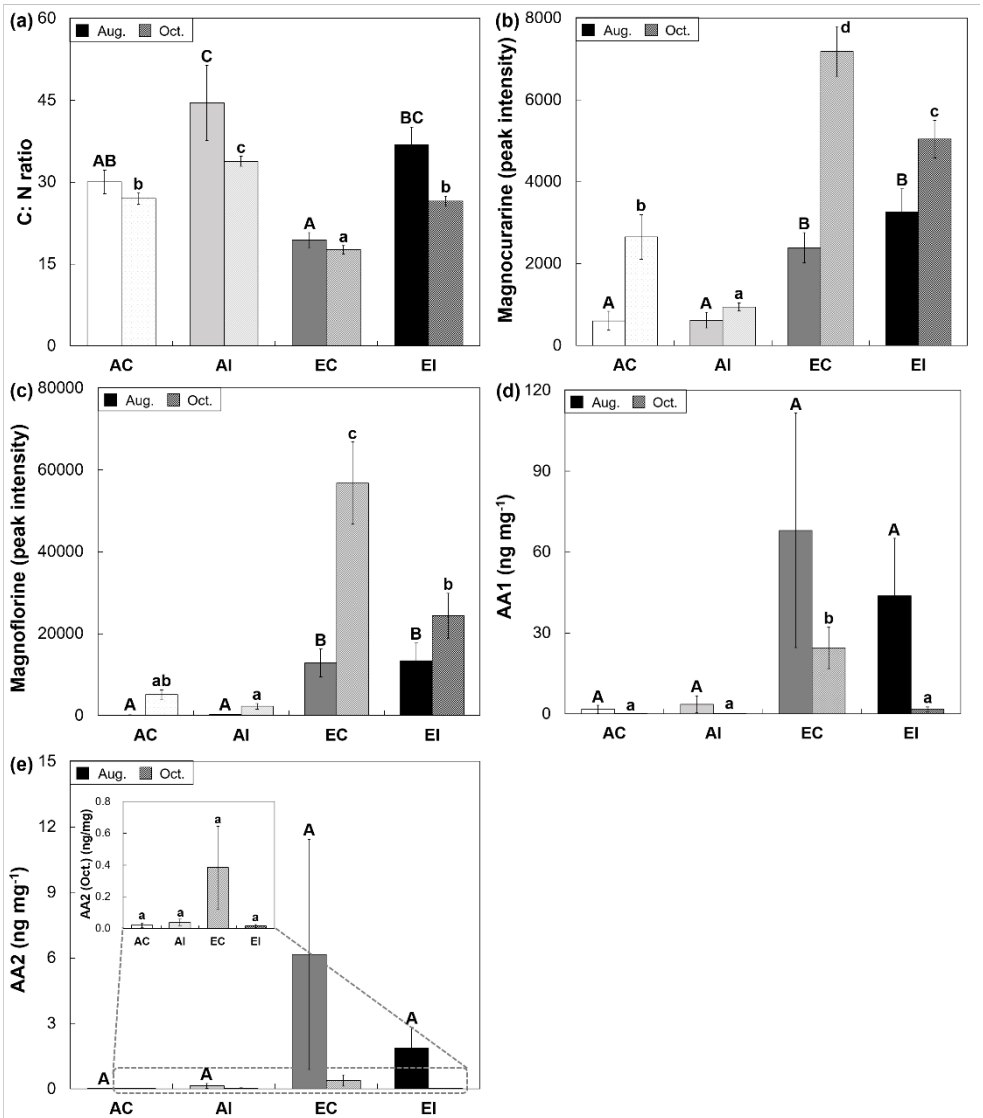


Figure 3-14. Elevated CO_2 concentration made the nutrient value low and the resistance high, but increased watering frequency reduced the impacts of elevated CO_2. C: N ratio (a), the peak intensity of magnocurarine (b), magnoflorine (c), and contents of aristolochic acid 1 (AA1) (d) and aristolochic acid 2 (AA2) (e) for each experimental group in August and October (n = 7, 15 for each group in August and October). The vertical bars show the standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test (p < 0.05).

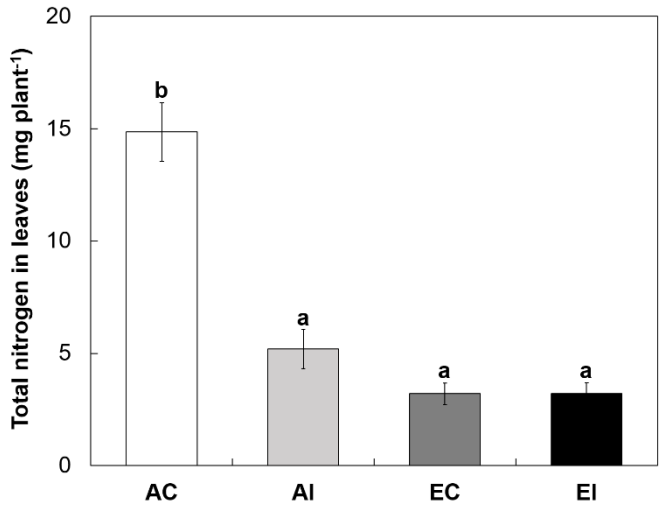


Figure 3-15. Nitrogen limitation did not occur for whole experimental period, based on total nitrogen weight in leaves. Total nitrogen weight was calculated as a multiple of total leaf dry weight and TN ratio in leaves for each plant, and that of each experimental condition was shown (n = 15 for each group). Vertical bars show standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test (p < 0.05).

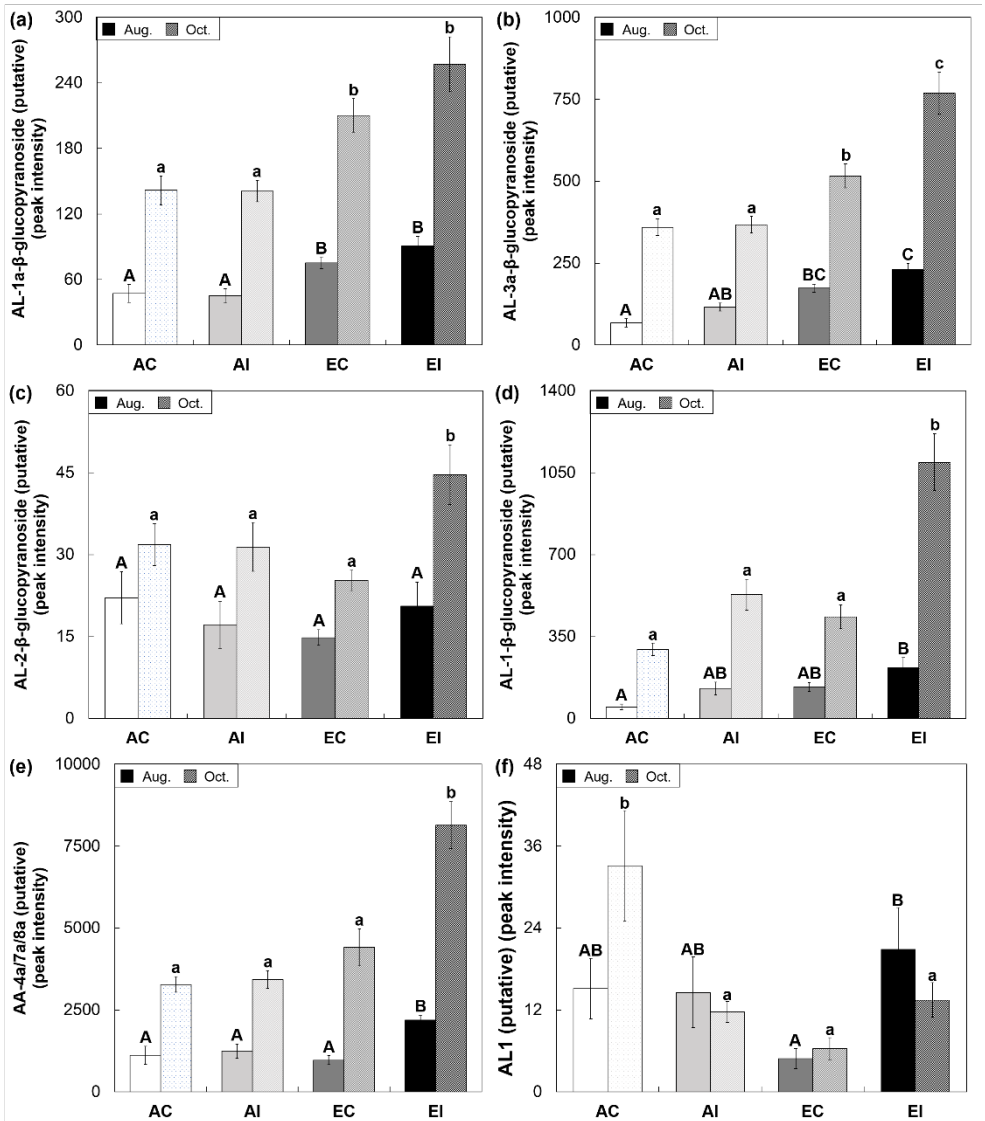


Figure 3-16. Other secondary metabolites did not show clear pattern according to CO_2 level and watering frequency. Peak intensity of AL-1a-β-glucopyranoside (putative) (a), AL-3a-β-glucopyranoside (putative) (b), AL-2-β-glucopyranoside (putative) (c), AL-1-β-glucopyranoside (putative) (d), AA-4a/7a/8a (putative) (e), AL1 (putative) (f) for each experimental group in August and October (*n* = 7, 15 for each group in August and October, respectively). Vertical bars show standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test ($p < 0.05$).

3.4. Discussion

Ecological mismatches between plants and insects have been regarded as one of the most considerable effects of climate change (Delucia et al., 2012). However, here I found no significant differences in phenological characteristics of the host plant, particularly in the emerging timing of shoots in the following year (Fig. 3-17a). Initial growth speed of shoots in the second year was consistently lower under the elevated CO_2 condition than the ambient CO_2 condition in the following year (Fig. 3-17b), indicating the possibility of the delayed initial development of host plants under climate change. Considering the fact that phenologies of insects are not generally affected by CO_2 elevation (Buse and Good, 1996; Yin et al., 2009), there might be little asynchrony in host plants and specialist herbivores in early life cycle of plant as the climate change progresses.

Nonetheless, rather than phenological characteristics, growth characteristics of the host plants seemed to be greater factors responsible for changes in plant-herbivore interaction under climate change. Both elevated CO_2 concentration and increased watering frequency acted as stresses to the host plants of dragon swallowtail butterflies, impeding the aboveground biomass production (Fig. 3-9a). Most of all, since the pot size was big enough to provide sufficient spaces for the most largely grown plants, these results did not seem to occur by the limitation of belowground growth, but rather by the experimental treatment. Between two experimental conditions assayed, the effect of CO_2 concentration was much stronger than that of watering frequency (Table 3-2), and increased CO_2 reduced both the

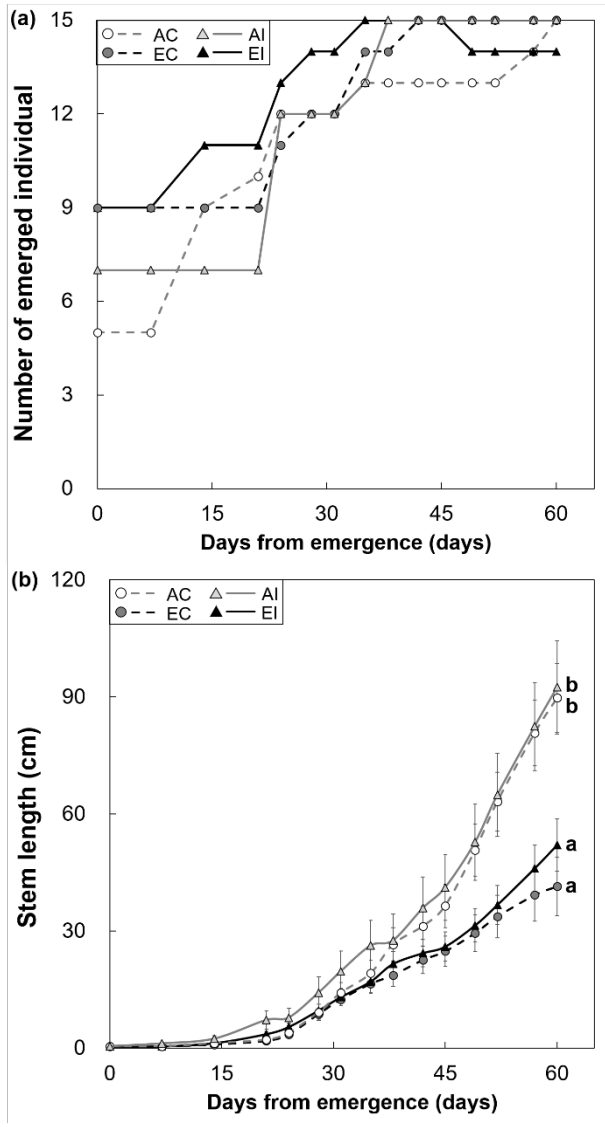


Figure 3-17. No differences in emerging timing of shoots in second year was observed, but initial growth speed in second year had clear differences according to CO_2 concentration. Number of emerged individuals in second year (a) and stem length of second year *A. contorta* (b) in 2020. Vertical bars show standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test ($p < 0.05$).

leaf and stem dry weights of the plants, which are quantitatively important for its specialist herbivores (Fig. 3-9b, c). This effect of elevated CO_2 was repeated in the same experiments for two years (Fig. 3-10). That is, although this result is contrary to recent researches, it is likely to be related to species-specific characteristics, neither to the artifact nor coincidence. The effects of elevated CO_2 concentration on the biomass production of the host plants shown in this study seemed to be related to their photosynthesis ability. Acclimation of plants to high CO_2 concentrations for a long period (from week to month) could inhibit the regeneration of chlorophyll (Delucia et al., 1985; Wullschleger et al., 1992) and accelerate the chlorophyll degradation through excessive starch accumulation in chloroplast (Bowes, 1991), resulting in a decrease in chlorophyll content. In addition, the regeneration and Rubisco activity could be inhibited by high CO_2 concentrations (Bowes, 1991), which could cause poor photosynthesis ability (Drake et al., 1997; Long, 1991). In this study, the chlorophyll content of *A. contorta* decreased more in elevated CO_2 concentrations (Fig. 3-12a). Additionally, after a month exposure to the experimental treatments, lower V_{cmax} values were also measured in the two elevated CO_2 concentration groups (EC and EI) compared to the two ambient CO_2 concentration groups (AC and AI) (Fig. 3-12b), whereas the J_{max} value was not different among the experimental groups (Fig. 3-12c). That is, *A. contorta* exhibited poorer photosynthesis under elevated CO_2 concentrations than under ambient CO_2 concentrations, not because of the electron transport capacity but because of lower Rubisco activity.

Despite the fact that increased watering frequency could be a constraint for plant growth, its effect seemed to differ according to the CO_2

concentration. Increased watering frequency caused negative effects under ambient CO_2 levels, whereas it had positive effects on leaf dry weight production and photosynthetic characteristics under elevated CO_2 levels (Figs. 3-9b, 12). This result is consistent with that of Ge et al. (2012), which showed less reduction of Rubisco activity under elevated CO_2 levels with high water availability in *Phalaris arundinacea*. Under ambient CO_2 concentrations, stress from increased watering frequency could be the main constraint to plants that adapt to low soil water content. However, since plants can show higher water use efficiency under high CO_2 conditions than ambient CO_2 conditions, sufficient or excessive water availability could be a partial compensatory factor for suppressed photosynthetic ability under elevated CO_2 conditions (Samarakoon and Gifford, 1995). That is, an increased watering frequency may ameliorate the negative effect of elevated CO_2 concentrations while it interrupts plant growth under ambient CO_2 concentration.

In addition to the growth performance related to herbivore food quantity, the physiological characteristics of plants that are responsible for herbivore food quality were also affected by elevated CO_2 concentrations and increased watering frequency. Like the leaf dry weight and AGDW, elevated CO_2 concentrations showed negative effects on leaf quality, indicated by the reduced growth and feeding performance of the specialist herbivores (Fig. 3-13, Table 3-2). This variation in food quality was likely to be closely related to the defensive secondary metabolites (Fig. 3-14) responsible for plant resistance against herbivores. In fact, according to the nutrient dilution hypothesis, plants under elevated CO_2 concentrations can show a higher C:

N ratio because of stimulated photosynthesis and consequent larger amounts of photosynthetic products (Taub et al., 2008). This increased C: N ratio by elevated CO_2 concentration could cause negative effects on specialist herbivores since it is much more important than the exact amounts of protein or sugars (Machado et al., 2015). Oppositely, regardless of the ratio, increased amounts of sugars could have positive effects on some herbivores such as garden snails (Llugany et al., 2019). Thus, it could be suggested that the detailed effects of increased C: N ratio on herbivores under high CO_2 are needed to be examined further. Despite those variable effects of larger value of C: N ratio under elevated CO_2 condition, the C: N ratio of plants grown under high CO_2 levels was lower in this study (Fig. 3-14a), probably resulted from both low content of carbohydrate due to the inhibition of photosynthesis and high content of nitrogen-containing secondary metabolites due to increased resistance. Thus, rather than the C: N ratio, the increased resistance of host plants with increased defensive secondary metabolites, particularly magnocurarine and magnoflorine (McKenzie and Price, 1953; Tahir, 1991), seemed to be responsible for the decrease in herbivore growth performance and consumed leaf area (Figs. 3-13, 3-14b-e). Indeed, several studies have reported increased secondary metabolites of plants under elevated CO_2 concentrations (Johnson and Hartley, 2017; Xu et al., 2019). Consistently, *A. contorta* seemed to show similar responses to high CO_2 levels in this study. As a consequence, the increased resistance with higher contents of defensive secondary metabolites would interrupt the consumption of the plant by herbivores (Schädler et al., 2007). Therefore, based on these effects of high CO_2, lower

leaf quality with increased resistance might occur under elevated CO_2 concentrations.

In contrast, increased watering frequency reduced the negative effects of elevated CO_2 concentrations on leaf quality without an opposing effect according to the CO_2 level, as shown by the growth performance of plants (Figs. 3-13, 14, Table 3-2). Additionally, the effect of watering frequency on the C: N ratio and secondary metabolites was much clearer at the end of the growth period of the plants (Fig. 3-14, Table 3-2). The effect of watering frequency is thought to be related to secondary metabolite synthesis. Several studies have shown that plants growing in dry conditions or experiencing drought stress exhibited increased secondary metabolite synthesis (Akula and Ravishankar, 2011), associated with the activation of gene expression involved in secondary metabolism (Yuan et al., 2012). Despite the fact that the detailed mechanisms are still largely unknown, it is obvious that dry soil conditions can enhance the synthesis of secondary metabolites in plants (Yang et al., 2018). Considering these facts, sufficient water availability provided by increased watering frequency could decrease the defensive secondary metabolite concentration and consequently increase the C: N ratio. Moreover, this effect of watering frequency on secondary metabolism would be clearer in the late growth period of the plants, when those compounds accumulate in plants. Particularly for specialist herbivores, since they could not be affected by the host plants' secondary metabolites to which they already adapted (Mathur et al., 2011), the cumulative effect might be much stronger when the accumulated compounds are different from those to which the plant previously adapted. Indeed, except

for aristolochic acid, the secondary metabolites of *A. contorta* increased in this study and the increasing patterns were most apparent in magnocurarine and magnoflorine, which may play a major role in the resistance to *S. montela* (Fig. 3-14, 3-16). This indicates that increased watering frequency could partially compensate for the negative effect of high CO_2 levels on both nutrient value and resistance to herbivores. Further research is necessary, focusing on how dry conditions increase or wet conditions decrease the secondary metabolite synthesis of plants.

3.5. Conclusion

The results of this study are consistent with recent researches indicating that climate change could cause a decline in insects biodiversity. Interestingly, here I showed that climate change could decrease the diversity of insects by reducing both food quantity and quality, which is a different scenario from the nutrient dilution hypothesis (Pennisi, 2020). In this study, elevated CO_2 concentrations and increased watering frequency affected not only plant growth but also plant secondary metabolites, showing different effects according to plant organs and growth stages. Overall, elevated CO_2 concentrations inhibited photosynthesis and reduced aboveground biomass production, especially in leaves closely related to food quantity for herbivores. In addition, high CO_2 levels increased the resistance of plant leaves, indicating diminished food quality for herbivores. Watering frequency also reduced food quantity by interrupting leaf increments, but it seemed to partially ameliorate the negative effects of elevated CO_2 concentrations on leaf quality by decreasing the secondary metabolite contents. Nonetheless,

since the effect of CO_2 concentrations appeared to be more significant, it could be suggested that both food quantity and quality would be reduced as climate change progresses. Consequently, the performance of a specialist herbivore might be threatened by global climate change as a result of these environmental effects on plants. Therefore, as Johnson et al. (2020) suggested, it is necessary to be aware of the possibility of different scenarios from the nutrient dilution hypothesis by carbon fertilization. Particularly, the suggested possible scenario in this study needs to be considered for effective and reasonable environmental management to conserve future biodiversity. Additionally, considering its dynamic effects on plants, watering frequency should be considered for precise prediction and understanding of the variations in plant-herbivore interactions under global climate change, particularly in the context of biodiversity conservation.

Chapter 4. Seasonality of host plant responses to climate change and consequent effects on plant-herbivore interactions

4.1. Introduction

Plants form many interactions with other organisms in ecosystem (Austin and Ballaré, 2014). Among those interactions, plant-herbivore interactions are one of the most important relationships because of the value of insects in biodiversity and the proportion of herbivorous insects (van der Meijden, 1996; War et al., 2012). As climate change progresses, the plant-herbivore interaction can be altered in various ways (DeLucia et al., 2012). There are several estimates about the change of plant-herbivore interactions under climate change, and most of them commonly suggest that there would be negative effects of climate change on the insect community (Wagner, 2020). In particular, responses of host plants to altered environmental factors generally play important roles for occurrence of the negative effects of climate change on herbivorous insects (Aucott, 2019).

Host plant responses to climate change can be observed in various plant traits (Liancourt et al., 2013). Most of all, physiological variations related to nutrient value and/or herbivore resistance occur by elevated CO_2 and temperature (Veteli et al., 2002; Welti et al., 2020). Moreover, the preference of adult female insects for oviposition can be affected by the change in volatiles of host plants (Witzgall et al., 2005; Lee et al., 2016), and the development of larvae could also be determined by the variations of metabolites of host plant (Walter et al., 2012). That is, physiological

characteristics of host plants are pivotal for explanation of plant-herbivore interactions (Baldwin, 2001; Sabelis et al., 2007). Thus, physiological responses of host plants under climate change should be studied particularly for precise understanding of future plant-herbivore interactions.

Because of this importance, numerous numbers of researches about the physiological responses of host plants under climate change have been performed recently (Becklin et al., 2016; Fowler et al., 2019). However, temporal variations of plant responses have not yet been considered sufficiently within those researches. Interactions between host plants and herbivores occur in various growth stages of plants. Indeed, Lepidoptera emerges several times a year, so that they have a number of times of interactions with plants in a year (Singer and Parmesan, 2010). In the perspective of herbivores, the direction of changes in food quantity is mostly positive, since plant biomass generally increase during plant growth period. However, the direction of variations in food quality, closely related to nutrient value and plant resistance, is changeable according to primary and secondary metabolism (Chen, 2008). Thus, it could be more important to examine the food quality rather than food quantity for sophisticated estimates of causal effects on herbivore growth performance.

Besides, those two metabolisms have temporal dynamics (Pérez-Bueno et al., 2015; Yang et al., 2020), and those dynamics occasionally show a time difference even because of the dependency of secondary metabolism on primary metabolism (Obata, 2019). As these metabolisms are closely related to energy productions such as photosynthesis, the environmental changes can affect the tendency of seasonal variations in plant primary and secondary

metabolism (Alnsour and Ludwig-Müller, 2015). That is, not only physiological characteristics of plants but also the cascading effects on herbivore could be different according to season under climate change condition. Therefore, temporal dynamics of plant responses and herbivore growth performance must be considered for more reliable prediction of the change in plant-herbivore interactions under climate change.

Here, my study examined the seasonality of growth performance of specialist and generalist herbivore (*Sericinus montela* Gray and *Spodoptera exigua* Hubner, respectively) with the consideration of nutrient value, constitutive and induced defense of a native host plant (*Aristolochia contorta* Bunge) under CO_2 elevation and soil water content variation. I hypothesized the improvement of nutrient value based on the increase of primary metabolites would be the most significant in the middle of plant growing season when the photosynthesis is the most active. On the other hand, the enhancement of plant defenses based on the accumulation of secondary metabolites might be the most apparent in the late of plant growing season. Consequently, I also conjectured the largest growth performance of specialist herbivore would be observed in the middle of plant growing season, whereas growth performance of generalist herbivore might be the largest in the early of plant growing season when the host plant contains the least amount of secondary metabolites.

To verify these hypotheses, I established an elevated CO_2 concentration level of 540 ppm according to the representative concentration pathway (RCP) 4.5 scenario using open-top chambers. I also altered soil water content by watering pattern representing local predictions of precipitation

pattern (Cha et al., 2016). Nutrient value and plant constitutive defense were assessed by measuring primary and secondary metabolites in leaves, respectively. At the same time, I examined the dynamics in phytohormone and secondary metabolites after specialist herbivorous treatment to determine the induced defense in host plant. Finally, I evaluated the consequences of these responses of host plant in specialist and generalist herbivore by measuring growth performance. My findings are expected to enhance concrete comprehension of changes in interactions between host plant and specialist herbivore caused by climate change.

4.2. Material and Methods

4.2.1. Plant material

Since *A. contorta* germination generally occurs from the late of June to July, *S. montela* interacts over 2-year-old of *A. contorta* from April to June (Shin, 1974; Lee, 2003). Thus, in order to examine the effects of elevated CO_2 and increased watering frequency on the interaction of *S. montela* and *A. contorta* in June, I prepared 2-year-old plants which were grown from 2019 under the same experimental condition of this study. Fifteen seedlings were used for each experimental treatment. I used these plants for every experiment conducted in June.

For preparation of 1-year-old plants, the seeds of *A. contorta* were collected in March 2020 at Gapyeong-gun, Gyounggi-do, South Korea (N 37°34’54”, E 127°31’41”), and stored at 4°C under dry conditions. They

were sowed in April 2020 on mixed soil (sand: topsoil = 2: 1, v/v) with consideration of the soil texture of natural habitat (Park et al., 2019), and germinated in a greenhouse at Seoul National University, Seoul, Korea. Seedlings about 7 cm in shoot height after germination were selected as 1-year-old plants on June 16, 2020, and transplanted one each into a cylindrical pot (diameter in 12 cm, height in 12 cm) filled with a mixed medium of sand (Gang-morae 25 kg, Ecosand, Korea) and topsoil (Barokeo 50 L, Youngnongsa, Korea) (ratio of 2 to 1, v/v). I decided the ratio of mixed medium referring to the soil nutrient and texture of natural habitat (Park et al., 2019; Park et al., 2020). Sixty-four seedlings were used for each experimental treatment after a week of adaptation.

4.2.2. Experimental design

I utilized open-top chambers (OTCs) of which shape were hexagonal pillar in 1.3 m diameter and 1.1 m height (Messerli et al., 2015), and they were placed in a greenhouse with open walls in Seoul National University (N 37°27’33”, E 126°57’13”) to prevent unexpected effects of natural rainfall and provide about 40% of relative light intensity proper for plants (Park et al., 2019). Eight OTCs were prepared and 2 chambers were used for each experimental treatment respectively. Four experimental treatments were prepared with two CO_2 concentrations (ambient and elevated, hereafter A and E) and two watering frequencies (control and increased, hereafter C and I). CO_2 control systems were installed for elevated CO_2 chambers individually to regulate CO_2 concentration for whole experimental period automatically, which consist of a sensor-transmitter coupled with a CO_2

controller (0 – 2,000 ppm_{CO2}, SH-MVG260, Soha-tech, Korea), a solenoid valve, and a CO_2 gas tank (99.999%, 40 L). The target CO_2 concentration of the elevated CO_2 treatment was 540 ppm, following RCP 4.5 scenario (Thompson et al., 2011). The mean CO_2 concentration of the elevated CO_2 chambers was 558.3 ± 25.0 ppm (n = 4), and that of the ambient CO_2 chambers was 433.8 ± 14.5 ppm (n = 4) (Fig. 4-1). Temperature and relative humidity monitors (HOBO pro v2, Onset, USA) were placed at the center of every chamber, and those values were similar in all chambers for experimental period (Fig. 4-2). Pots were set on wooden plates to minimize the artificial effects of the wind that came out at the bottom of the chamber by ventilation system. Tap water was provided once a week in the control watering frequency treatment, whereas it was provided twice a week in the increased watering frequency treatment, using a sprinkling can. This pattern of watering frequency treatment was determined based on the research related to local precipitation prediction (Cha et al., 2016).

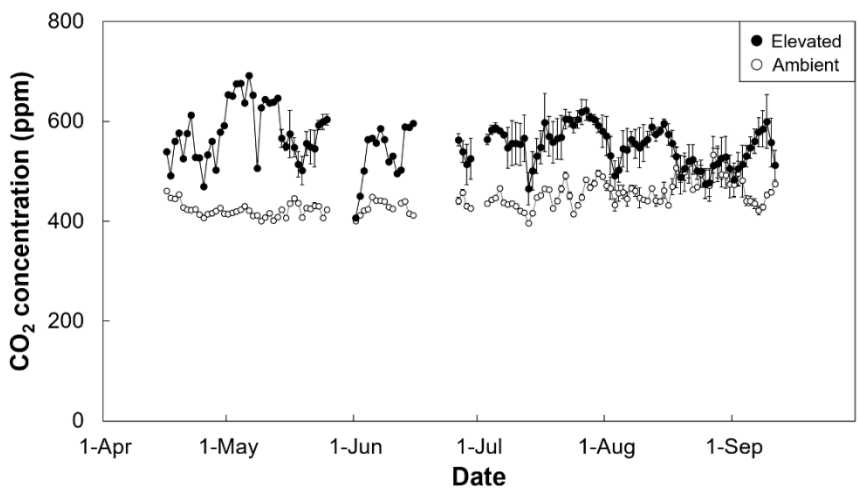


Figure 4-1. Daily average CO_2 concentration of elevated and ambient CO_2 chambers were distinct for the whole experimental period (n = 4 for each). The vertical bars show the standard error for each group.

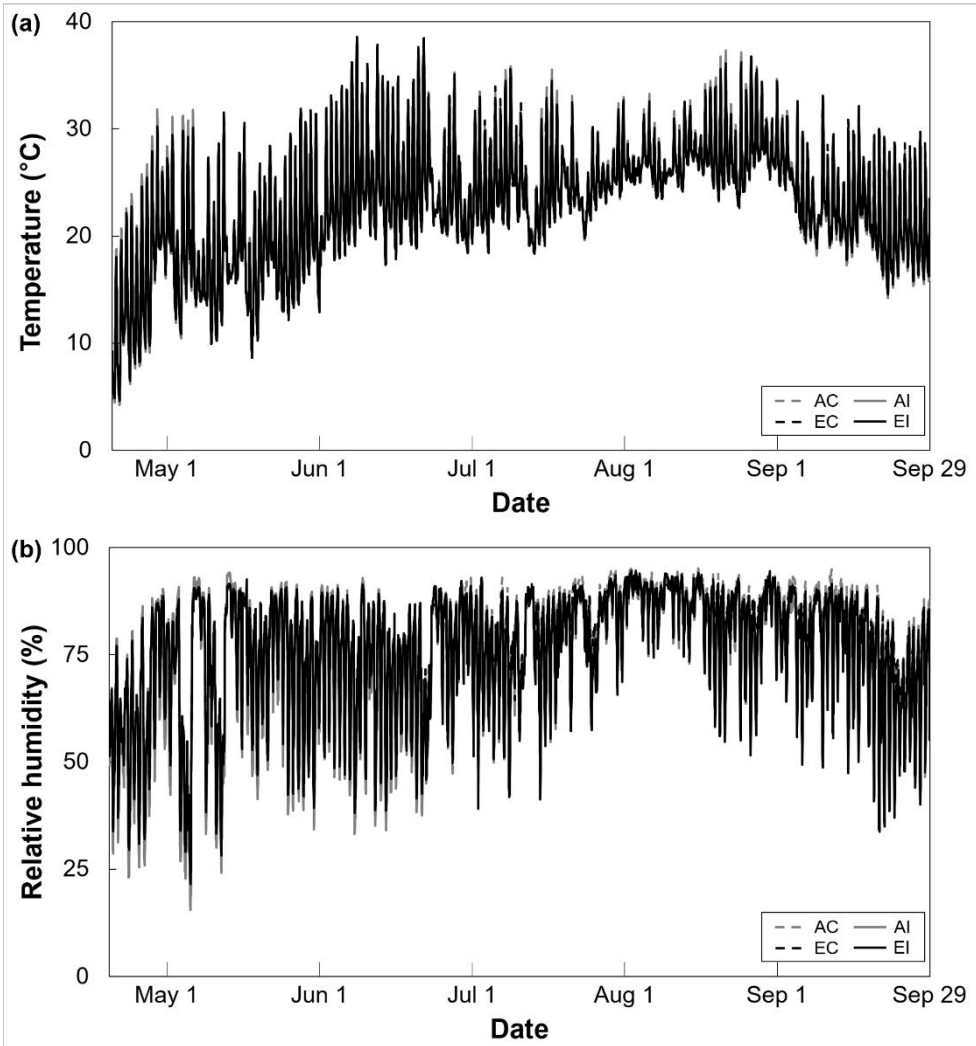


Figure 4-2. Temperature (a) and relative humidity (b) of each experimental condition (*n* = 2) were monitored and no significant difference was observed among experimental groups.

4.2.3. Measurement of relative growth performance of specialist and generalist herbivore

To examine seasonal variations in consequent effects of host plant response on specialist herbivore under climate change, I measured relative growth performance of *S. montela* for three times (June, July, and September 2020), referred to emergence timing of *S. montela* in nature (Shin, 1974). The eggs were collected at common gardens in Pyeongtaek-si, Gyounggi-do, Korea (N 37°04'06", E 127°00'27", in June and September) and Seoul National University (N 37°27'97", E 126°57'11", in July). They hatched after two or three days, and raised for three days more with sufficient *A. contorta* leaves and space to ensure that all larvae were in the second instar stage. The leaves were collected including petiole, and the petiole of each leaf was immersed in a 1.5 mL tube (Eppendorf, Germany) and sealed with parafilm (Bemis, USA) to keep leaves from dehydration. Supports for tubes were made of aluminum foil to make the larvae on the leaves move freely (Fig. 3-8). I selected a total of 120 larvae based on weight, and 30 larvae were used for each experimental treatment. Photos of all leaves of each treatment were taken before insertion, and they were used to calculate the leaf area consumed. Additional leaves were inserted every three days, when most of leaves were consumed over 50%. This bioassay was performed for six days, and the final weights of the larvae were measured at the end. The relative growth rate (RGR) was calculated as the ratio of the amount of increased weight to initial weight of each larva per day. The bioassays in June, July, and September were started on June 13th, July 17th, and September 1st, respectively.

In parallel, in order to evaluate the effects of induced plant defense to specialist herbivore under climate change, I conducted additional specialist herbivore bioassay with same process above, using the oral secretion (OS) from *S. montela* treated leaves. I used patterning wheels to damage about 20% of leaves, and then applied the OS solution which made of diluted OS into the ratio of 1 to 20 (McGale et al., 2018). These bioassays were performed in July and September 2020, started on July 20th, September 4th, respectively.

In addition to specialist herbivore bioassay, I also investigated relative growth performance of *S. exigua* known as a generalist herbivore tolerant to toxic secondary metabolites from *A. contorta* including aristolochic acids (Feng et al., 2012; Jeude and Fordyce, 2014), to compare the seasonality of plant-herbivore interactions under climate change between specialist and generalist herbivore. Considering higher lethality compared to specialist herbivore, I selected a total of 360 larvae of *S. exigua* in third instar stage, and used 90 larvae for each experimental treatment. Other detail methods of the bioassay of *S. exigua* was the same with that of *S. montela*. The bioassays were conducted three times (June, August, and September 2020) for three days each, in accordance with the emergence timing of *S. montela*. Those were started at June 17th, August 14th, and September 8th, respectively.

4.2.4. Carbon and nitrogen analysis

To assess the food quality of host plant leaves, I examined the leaf C: N ratio as an estimate through stoichiometric analysis of the total carbon

(TC) and total nitrogen (TN) ratio in the leaves of the plants. The plant leaves were dried at 60°C in a dry oven and ground (Wiley Mini-Mill 3380L10, Thomas, USA) to make homogeneous mixtures. The TC and TN contents were measured using an elemental analyzer (Flash EA 1112, Thermo Electron, USA) at the National Instrumentation Center for Environmental Management (NICEM) at Seoul National University.

4.2.5. Primary metabolites measurement

I further measured primary metabolites to evaluate the nutrient value of host plant leaves. For primary metabolites extraction, 10 mg of freeze-dried *A. contorta* leaf of fourth and fifth from the top was separately weighted in each 1.5 mL tube. 1 mL of methanol (HPLC grade, Sigma-Aldrich, USA) was added to extract metabolites. The samples were vortexed for 40 sec and sonicated for 40 min. The supernatants were collected sand filtered through 0.45 μm PTFE syringe filters after sonication. 100 mL of each filtered sample was transferred into amber GC vials and dried with nitrogen gas for 5 min in an evaporator. After that, I performed the oximation process with following solutions: 30 μl of 20,000 $\mu g \cdot mL^{-1}$ methoxylamine hydrochloride in pyridine (Sigma-Aldrich, USA), 50 μl of N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (Sigma-Aldrich, USA), and 10 μl of 300 $\mu g \cdot mL^{-1}$ of 2-chloronaphthalene (Sigma-Aldrich, USA) in pyridine as an internal standard (IS). The samples were incubated in a 65°C heat block for 1 hour.

These extractions were analyzed using a Shimadzu gas chromatography system (GC-MS QP2020, Shimadzu Corp., Japan). The

GC-MS condition of metabolites was referred to Suh et al. (2013) with minor modifications (Appendix 3). The temperature of GC inlet was wet to 250°C with an injection volume of 1.0 μL and a split ratio of 1:5, using helium as the carrier gas at a constant-flow rate of 1.0 $mL \cdot min^{-1}$. The range of mass was set between 50 and 600 Da. A Rtx-5MS column (Shimadzu, Corp., Japan) with 30 m × 0.25 mm I.D. × 0.25 μm d_f dimensions was used for the analysis. The oven program was set to 70°C, held for 5 min, and then ramped up to 130°C at $15°C \cdot min^{-1}$. After that, a condition of temperature of 160°C at $4°C \cdot min^{-1}$ was held for 15 min, and 300°C at $10°C \cdot min^{-1}$ was held for 9 min. A total of running time was 54.5 min. National Institute of Standards and Technology (NIST) mass spectral search library was used for identification of metabolites in samples, and a match quality greater than 80% was selected in the peak assignment. Finally, normalization was performed by dividing the peak area of each compound by that of the IS to compare relative abundance of selected metabolites in each sample.

4.2.6. Phytohormone and secondary metabolites analysis

To examine the constitutive and induced defensive responses in host plants to specialist herbivore, I measured the contents of phytohormone and secondary metabolites before and after OS treatment. Sixth to tenth leaf from the top were selected as samples, and a half of every leaf was collected before OS treatment as a control. The rest of leaves were damaged by a patterning wheel, and the diluted OS solution (1/20 diluted in distilled water) was applied. Two or three half-leaves were collected for phytohormone and secondary metabolites analysis after 1 hour and 2 days,

respectively. These samples were collected from twelve representative plants for each condition in June, July, and September 2020. After collection, I immediately froze the leaves in liquid nitrogen and stored them at -80°C, and ground in a frozen state before extraction.

To extract phytohormone, I used ethylacetate (HPLC grade, Sigma-Aldrich, USA) spiked with labeled phytohormones as IS: D_6-ABA, D_4-SA, and JA-$^{13}C_6$-Ile in 20 nguL-1. Two steel balls of 3 mm diameter and 1 mL of spiked ethylacetate were added for each 50 mg of ground *A. contorta* leaf samples, and vortexed using Tissuelyser II (Qiagen, Germany) with 26 stroke/sec-1 for 2 min. Samples were centrifuged at 13,000 rpm for 20 min at 4°C, and supernatants were transferred to new 2 mL tube each (Eppendorf, Germany). This extraction process was repeated by adding 0.5 mL of ethylacetate without IS. Supernatants were combined after centrifuge at the same condition, and evaporated using HyperVAC-MAX (Hanil Scientific Inc., Korea) until dryness at 30°C. After confident dryness, 500 uL of 70% methanol (HPLC grade, Sigma-Aldrich, USA) were then added to dissolve metabolites, and vortexed with Tissulyser II with 26 stroke/sec-1 for 1 min. 400 uL of dissolved samples were transferred into a HPLC vial each, after centrifuge at 13,000 rpm for 10 min at 4°C. The extracts were analyzed by UHPLC-HESI-MS/MS, referring to Schäfer et al. (2016) with little modifications (Appendix 4).

Additionally, for secondary metabolites extraction, 1 mL of 40% methanol (HPLC grade, Sigma-Aldrich, USA) containing 0.1 $\mu g \cdot mL^{-1}$ of tribenzylamine (TBA) (≥ 99.0%, Sigma, USA) as an internal standard was added to 50 mg of the ground sample. The secondary metabolites were

measured in the extraction using an ultra-performance liquid chromatography (UPLC)-quadrupole orthogonal time of flight mass spectrometer (qTOFMS) (Waters ACQUITY UPLC, Micromass Q-Tof micro, Waters, USA), following the method of Mao et al. (2017) with minor modifications (Appendix 5).

4.2.7. Statistical analysis

First of all, the normality of all data was checked by Shapiro-Wilk test. Then, I conducted two-way analysis of variance (ANOVA) to examine the effect of elevated CO_2 and increased watering frequency on measured variables. After that, the differences between the experimental groups were checked by Duncan's post-hoc test. The significance of changes according to seasons were confirmed by *t*-test. These tests were performed at the 0.05 significance level using SPSS (ver. 22.0). In addition, I also conducted principal component analysis to examine the seasonality and to figure out significant differences of primary and secondary metabolites using R version 3.6.3 (R Core Team, 2019).

4.3. Results

4.3.1. Different seasonal variations in relative growth performance of specialist and generalist herbivore

Relative growth performance of specialist and generalist herbivore showed distinct seasonal variations (Fig. 4-3). In the case of specialist herbivore, the RGR of AI and EC showed the significantly highest and lowest values respectively regardless of season, but the extent of differences among experimental groups changed according to season (Fig. 4-3a). The values of all experimental groups in July showed significant differences because of large values in two increased watering frequency groups (AI and EI) (Table 4-1), but the differences among experimental groups in September were less than July since the RGR of AI and EI decreased. The values of all experimental groups did not differ largely in June. Compared to AI and EI, the RGR of two control watering frequency groups (AC and EC) were relatively low in July, and decreased in September with larger differences in EC than AC. Despite the fact that the differences in RGR among experimental groups showed the seasonal variations, the patterns were consistent throughout the whole experimental period.

To understand the induced defensive responses of host plant to specialist herbivore under climate change, I examined relative growth performance of specialist herbivore when OS-treated leaves were provided (Fig. 4-3b). Most of all, the values of RGR with OS-treated leaves were lower than those with normal leaves in all experimental groups (Fig. 4-3a,

b). In July, the highest and second highest values of RGR were obtained under AC and AI respectively, and lower RGR values were observed under two elevated CO_2 concentration groups (EC and EI). RGR increased for all experimental groups in September compared to July, but there was no significant statistical difference among groups (Table 4-1).

In contrast, the patterns of relative growth performance of generalist herbivore were different from those of specialist herbivore (Fig. 4-3c). They showed the most active growth performance in June with significant increases from August, and the highest and lowest RGR values in EC and AI respectively, unlike the patterns of specialist herbivore. However, in August, the negative RGR values were obtained in all experimental groups. In September, there were slight increases of RGR in all groups, but overall growth was lower than June.

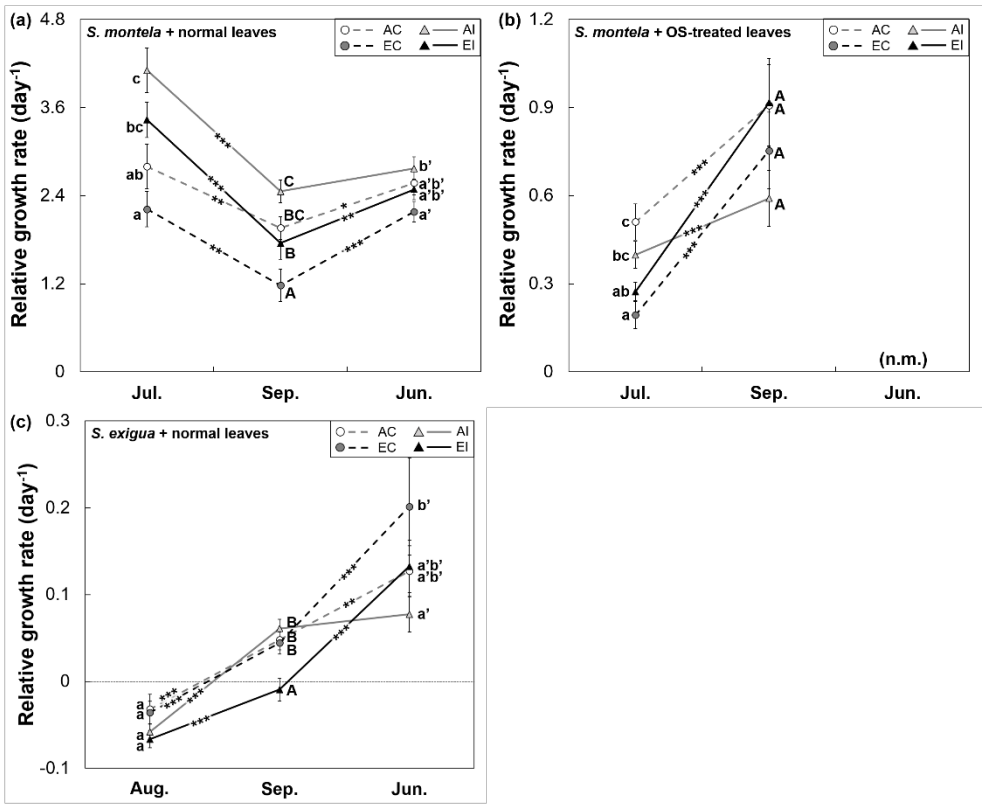


Figure 4-3. Relative growth performance of *S. montela* (specialist herbivore) and *S. exigua* (generalist herbivore) showed different seasonal variations according to experimental conditions. Relative growth rate (RGR) of *S. montela* with normal leaves (a) and OS-treated leaves (b) and that of *S. exigua* with normal leaves (c) of each experimental group (*n* = 30, 30, 30, respectively). The vertical bars show the standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test ($p < 0.05$). (AC: ambient CO_2 concentration and control watering frequency; AI: ambient CO_2 concentration and increased watering frequency; EC: elevated CO_2 concentration and control watering frequency; EI: elevated CO_2 concentration and increased watering frequency; n.m.: not measured)

Table 4-1. *F*-values and *p*-values from two-way analysis of variance (ANOVA) results of the effects of CO_2 concentration and watering frequency on primary and secondary metabolites of *A. contorta*, and herbivores' (*S. montela* and *S. exigua*) growth performance of in an OTC experiment. Degrees of freedom (*df*) of all experimental treatments were 1. Statistically significant effects are represented in boldface. (RGR: relative growth rate; JA: jasmonic acid)

		CO_2		Watering frequency		CO_2 × Watering frequency	
		F	*p*	*F*	*p*	*F*	*p*
Herbivore growth performance	RGR of *S. montela* with normal leaves (Jun.)	**4.180**	**0.043**	2.306	0.132	0.104	0.747
	RGR of *S. montela* with normal leaves (Jul.)	**5.904**	**0.017**	**24.05**	**0.000**	0.033	0.855
	RGR of *S. montela* with normal leaves (Sep.)	**14.74**	**0.000**	**7.731**	**0.007**	0.042	0.839
	RGR of *S. montela* with OS-treated leaves (Jul.)	**16.61**	**0.000**	0.297	0.587	3.008	0.086
	RGR of *S. montela* with OS-treated leaves (Sep.)	0.441	0.509	0.350	0.556	3.451	0.067
	RGR of *S. exigua* with normal leaves (Jun.)	**4.993**	**0.038**	1.047	0.320	0.627	0.439
	RGR of *S. exigua* with normal leaves (Jul.)	0.178	0.674	3.696	0.057	0.026	0.873
	RGR of *S. exigua* with normal leaves (Sep.)	0.293	0.590	0.055	0.815	**6.140**	**0.015**
C:N ratio	C: N ratio (Jun.)	1.599	0.213	2.101	0.154	0.941	0.337
	C: N ratio (Jul.)	0.184	0.671	4.079	0.051	**5.354**	**0.026**
	C: N ratio (Sep.)	**13.50**	**0.001**	**18.21**	**0.000**	0.645	0.426
Primary metabolites	Contents of soluble sugars (relative abundance) (Jun.)	3.753	0.059	0.354	0.555	0.261	0.612
	Contents of soluble sugars (relative abundance) (Jul.)	1.018	0.325	**19.66**	**0.000**	1.078	0.312
	Contents of soluble sugars (relative abundance) (Sep.)	0.603	0.442	3.223	0.079	0.520	0.475
	Contents of free amino acids (relative abundance) (Jun.)	3.330	0.075	**7.136**	**0.011**	1.126	0.295
	Contents of free amino acids (relative abundance) (Jul.)	2.205	0.153	**4.692**	**0.043**	1.741	0.202
	Contents of free amino acids (relative abundance) (Sep.)	5.593	0.028	14.78	0.000	2.533	0.119
Secondary metabolites	Total secondary metabolites (normalized peak intensity) (Jun.)	**23.61**	**0.000**	0.182	0.672	0.272	0.605
	Total secondary metabolites (normalized peak intensity) (Jul.)	**9.863**	**0.003**	3.864	0.056	1.442	0.236
	Total secondary metabolites (normalized peak intensity) (Sep.)	**27.51**	**0.000**	**13.02**	**0.001**	1.033	0.315

Table 4-1. (continued.)

		CO_2		Watering frequency		CO_2 × Watering frequency	
		F	*p*	*F*	*p*	*F*	*p*
Secondary metabolites	Magnocurarine (normalized peak intensity) (Jun.)	**15.13**	**0.000**	0.883	0.354	**5.170**	**0.030**
	Magnocurarine (normalized peak intensity) (Jul.)	**19.13**	**0.000**	**5.621**	**0.022**	1.695	0.200
	Magnocurarine (normalized peak intensity) (Sep.)	1.355	0.251	**20.34**	**0.000**	0.123	0.728
	Magnoflorine (normalized peak intensity) (Jun.)	**23.34**	**0.000**	0.194	0.663	0.286	0.597
	Magnoflorine (normalized peak intensity) (Jul.)	**18.64**	**0.000**	**4.848**	**0.033**	1.432	0.238
	Magnoflorine (normalized peak intensity) (Sep.)	**13.57**	**0.001**	**10.90**	**0.002**	3.483	0.069
	Aristolochic acid 1 (ng mg^{-1}) (Jun.)	**10.56**	**0.003**	0.371	0.547	2.568	0.119
	Aristolochic acid 1 (ng mg^{-1}) (Jul.)	0.203	0.654	0.276	0.602	0.309	0.581
	Aristolochic acid 1 (ng mg^{-1}) (Sep.)	**63.22**	**0.000**	0.014	0.908	1.196	0.280
	Aristolochic acid 2 (ng mg^{-1}) (Jun.)	**7.931**	**0.008**	2.042	0.163	3.495	0.071
	Aristolochic acid 2 (ng mg^{-1}) (Jul.)	0.009	0.925	0.334	0.566	0.502	0.482
	Aristolochic acid 2 (ng mg^{-1}) (Sep.)	3.599	0.064	0.540	0.466	3.059	0.087
Phytohormone	Contents of JA before OS treatment (ng g^{-1}) (Jun.)	**4.692**	**0.038**	**5.207**	**0.029**	3.810	0.060
	Contents of JA before OS treatment (ng g^{-1}) (Jul.)	0.000	0.993	0.030	0.864	1.393	0.244
	Contents of JA before OS treatment (ng g^{-1}) (Sep.)	2.657	0.110	**6.847**	**0.012**	**10.49**	**0.002**
	Contents of JA after OS treatment (ng g^{-1}) (Jun.)	0.127	0.724	2.166	0.151	0.827	0.370
	Contents of JA after OS treatment (ng g^{-1}) (Jul.)	0.001	0.970	**9.715**	**0.003**	**7.154**	**0.010**
	Contents of JA after OS treatment (ng g^{-1}) (Sep.)	**6.729**	**0.013**	**14.30**	**0.000**	**7.442**	**0.009**

4.3.2. Seasonal dynamics in leaf nutrient value related to unconformable variations in soluble sugars and free amino acids

To evaluate the nutrient value of *A. contorta* leaves as one of the estimates of food quality for *S. montela*, C: N ratio of *A. contorta* leaves were analyzed according to season (Fig. 4-4). C: N ratio values of all experimental groups in July were the lowest among seasons, and they all increased twice in September with the highest value in AI. The values were similar between September and June, but the differences among groups were not statistically significant only in June. The highest C: N ratio was observed in AI in both July and September, but the lowest value was obtained in EC. AC and EI showed intermediate level of C: N ratio.

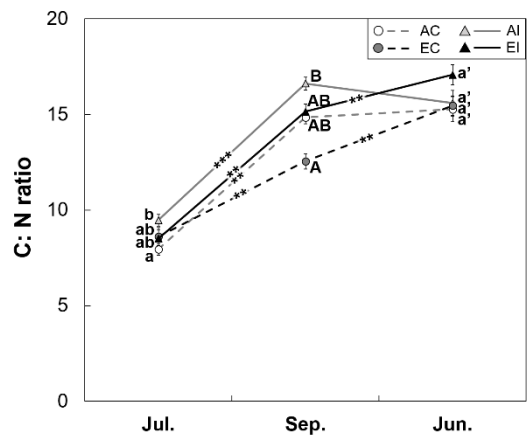


Figure 4-4. Similar C: N ratio of *A. contorta* in June had decreased in July, but it re-increased in September with significant differences among experimental groups. The vertical bars show the standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test ($p < 0.05$). Asterisks on each line shows significances of differences between seasons according to *t*-test (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

In addition to C: N ratio, I also analyzed primary metabolites in *A. contorta* leaves to elaborate the seasonal variations in C: N ratio and leaf nutrient value in detail (Fig. 4-5). A total of 46 primary metabolites including fatty acids, soluble sugars, and free amino acids were detected. Most of all, primary metabolites of July were clearly distinct only, but June and September appeared to be similar according to the results of PCA. Linoelaidic acid, one of the fatty acids, had the highest score in PC1, followed by two other fatty acids and two free amino acid (Table 4-2).

Considering the soluble sugars and free amino acids are important nutritious compounds for herbivores, I calculated the total amount of them in detail. Although soluble sugars and most free amino acids did not show high score in PC1, they had clear differences according to seasons (Fig. 4-6). Under AC, the difference in soluble sugar content between July and September was not clear, whereas that in free amino acid content was statistically significant. Under AI and EI, both soluble sugar and free amino acid contents decreased in September, but the extent of decrease was higher in free amino acids than in soluble sugars. Soluble sugars continuously decreased in June, but free amino acids did not show significant differences. Contrastingly, the contents of soluble sugars in EC increased and decreased according to seasons, while free amino acids were always similar with the largest value among experimental groups in June.

Table 4-2. PC1 scores of 46 primary metabolites in the result of principal component analysis (PCA) according to seasons.

Rank	Metabolites	PC1	Rank	Metabolites	PC1
1	Linoelaidic acid	0.2372	24	Glyceric acid	0.1227
2	Alanine	0.2356	25	Glucose	0.1227
3	Aspartic acid	0.2346	26	Galactose	0.1192
4	α-Linolenic acid	0.2322	27	Trehalose	0.1148
5	Glycerol	0.2216	28	1-Triacontanol	-0.1147
6	Butanedioic acid	0.2149	29	Cellobiose	0.1081
7	Glycine	0.2138	30	Caffeic acid	0.1078
8	2-Keto-L-gulonic acid	0.2090	31	Stearic acid	0.0929
9	Palmitic acid	0.2088	32	Propanedioic acid	0.0916
10	Galactinol	0.2063	33	1-Monopalmitin	-0.0913
11	Threonic acid	0.2003	34	Lactic acid	0.0884
12	Myo-Inositol	0.1993	35	Ascorbic acid	-0.0764
13	2-Butenedioic acid	0.1871	36	α-Tocopherol	-0.0763
14	Ribitol	0.1818	37	β-Sitosterol	0.0648
15	Phosphoric acid	0.1734	38	Tryptophan	-0.0440
16	Sucrose	0.1719	39	Maltose	-0.0363
17	Malic acid	0.1643	40	Dopamine	0.0345
18	Tyramine	0.1625	41	1-Octacosanol	0.0233
19	Serine	0.1575	42	Threonine	0.0177
20	Valine	0.1524	43	Pinitol	-0.0159
21	Fructose	0.1319	44	Lactose	-0.0138
22	Glyceryl-glucoside	0.1299	45	Tyrosine	-0.0108
23	D-Allose	0.1280	46	Glutamic acid	0.0057

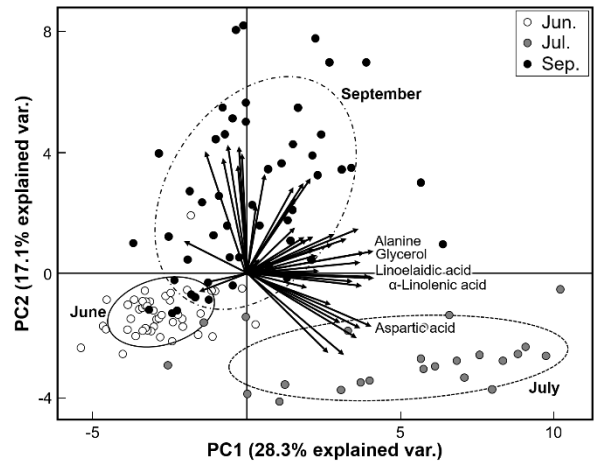


Figure 4-5. Primary metabolites of *A. contorta* were distinct between June and July according to principal component analysis (PCA). Six metabolites, which had three highest and lowest score of PC1, showed representatively.

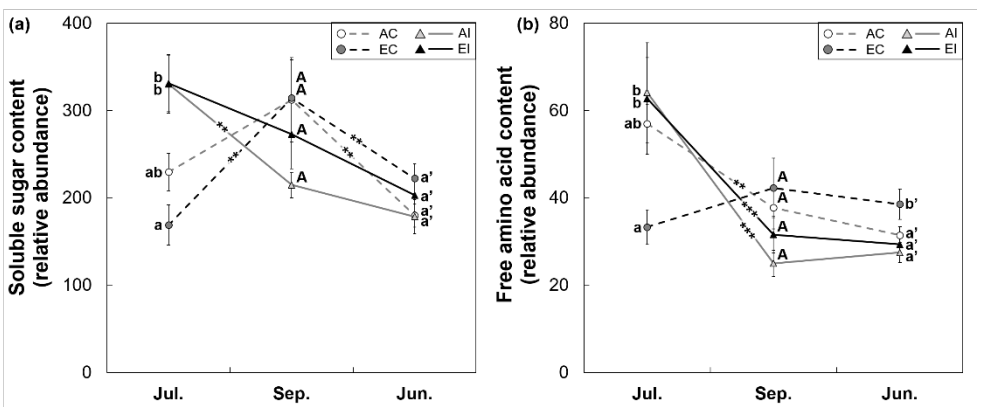


Figure 4-6. Both soluble sugars and free amino acids were increased in July, but the extent of increase were different according to metabolites and experimental conditions. Contents of soluble sugars (a) and free amino acids (b) in four experimental groups according to seasons. The vertical bars show the standard error for each group. Different letters indicate statistically different sub-groups by Duncan's post-hoc test ($p < 0.05$). Asterisks on each line shows significances of differences between seasons according to *t*-test (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

4.3.3. Increased secondary metabolites for constitutive defenses in late plant growth period

I further examined secondary metabolites to assess the plant constitutive resistance as another estimates of food quality for *S. montela*. Unlike primary metabolites, secondary metabolites related to herbivore defenses of September were the most distinct among three seasons in PCA (Fig. 4-7), with the highest contents (Fig. 4-8). Among 10 secondary metabolites, several aristolactam (AL) modified metabolites showed higher score of PC1 (Table 4-3). These metabolites were contained relatively higher concentration in elevated CO_2 groups (EC and EI), than in two ambient CO_2 groups (AC and AI) (Fig. 4-8). In addition, secondary metabolites increased in September under control watering frequency condition, whereas those did not change under increased watering frequency condition (Fig. 4-8). Among secondary metabolites, magnocurarine, magnoflorine, AA1, and AA2 were more abundant compared to other six metabolites (Fig. 4-9). These four metabolites were higher under elevated CO_2 groups, and control watering frequency groups.

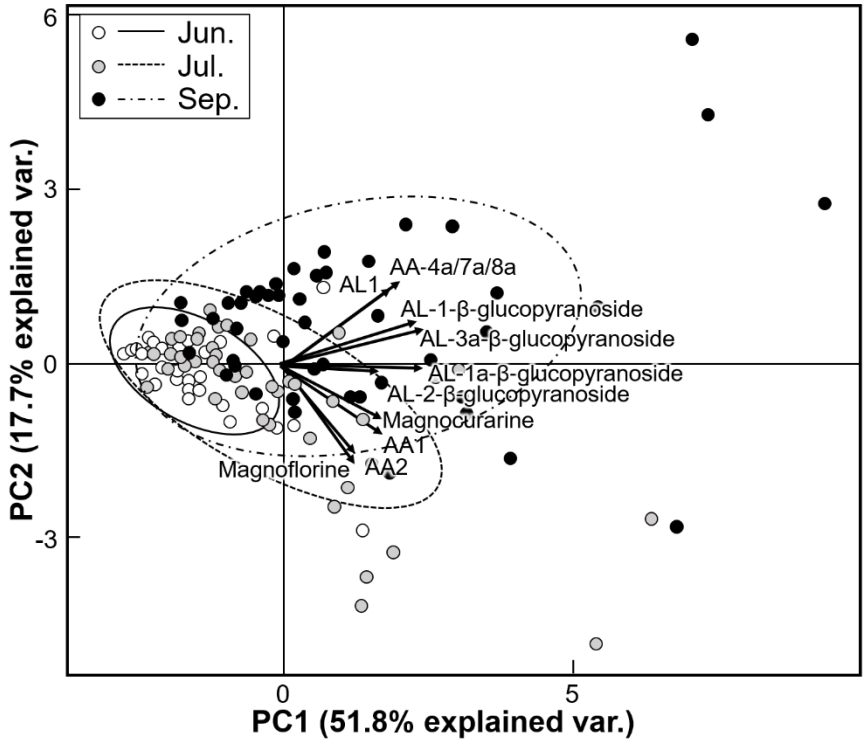


Figure 4-7. Secondary metabolites of September were the most distinct among three seasons, as a result of principal component analysis (PCA).

Table 4-3. PC1 scores of 10 secondary metabolites in the result of principal component analysis (PCA) according to seasons.

Rank	Metabolites	PC1
1	AL-3a-β-glucopyranoside	0.4094
2	AL-1a-β-glucopyranoside	0.4086
3	AL-1-β-glucopyranoside	0.3830
4	Aristolactam 1	0.3270
5	AA-4a/7a/8a	0.3078
6	Aristolochic acid 1	0.2823
7	AL-2-β-glucopyranoside	0.2757
8	Magnocurarine	0.2756
9	Magnoflorine	0.2090
10	Aristolochic acid 2	0.2043

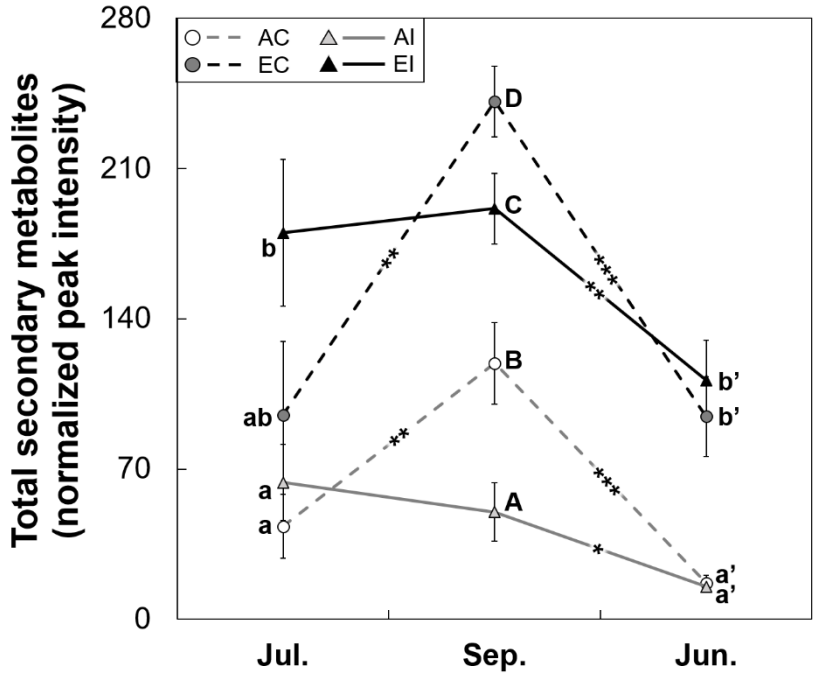


Figure 4-8. Total amount of secondary metabolites overall increased for whole growth period of plant, and the increase showed largely in EC and AC in September. Total amount of secondary metabolites of each experimental condition according to seasons. The vertical bars show the standard error for each group (*n* = 9, 12, 12 for each group in June, July, September, respectively). Different letters indicate statistically different sub-groups by Duncan's post-hoc test ($p < 0.05$). Asterisks on each line shows significances of differences between seasons according to *t*-test (*: $p < 0.05$; **: $p < 0.01$).

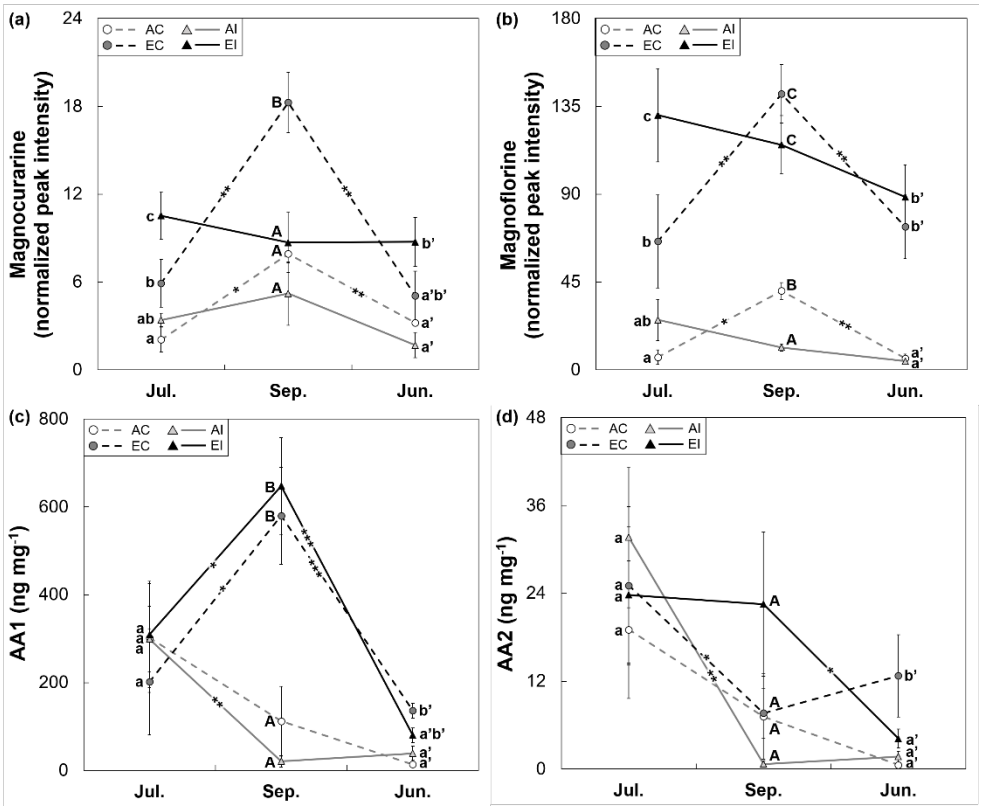


Figure 4-9. Four major secondary metabolites had different seasonal dynamics according to experimental condition. Contents of magnocurarine (a), magnoflorine (b), aristolochic acid 1 (AA1) (c), and aristolochic acid 2 (AA2) (d) of each experimental condition according to seasons. The vertical bars show the standard error for each group (*n* = 9, 12, 12 for each group in June, July, September, respectively). Different letters indicate statistically different sub-groups by Duncan's post-hoc test ($p < 0.05$). Asterisks on each line shows significances of differences between seasons according to *t*-test (*: $p < 0.05$; **: $p < 0.01$).

4.3.4. Seasonal dynamics of JA inducibility but no differences in induced responses in secondary metabolites according to plant growing season

In addition to constitutive defenses in plant, I also evaluated the induced defensive responses by measuring jasmonic acid (JA) inducibility and the differences of secondary metabolites after OS treating on leaves. Clear increases in JA were observed for the whole plant growing season, but there was significant difference among experimental groups in July and September (Fig. 4-10). The overall content of JA after OS treatment was the highest in July, with the largest increase in EC (Fig. 4-10b). JA content in September were similar with in June, but EI was classified into different statistical sub-group showing the highest content of JA (Fig. 4-10c). Two ambient CO_2 groups (AC and AI) showed intermediate values between those of two elevated CO_2 groups (EC and EI) in July, and they had no differences with EC in September. In contrast, no significant differences in defensive secondary metabolites among all seasons were observed (Fig. 4-11). Despite the fact that there was not any statistical significance, secondary metabolite increased after specialist herbivore attack the most in July, particularly the largest increase was showed in magnocurarine and magnoflorine in EI (Fig. 4-12).

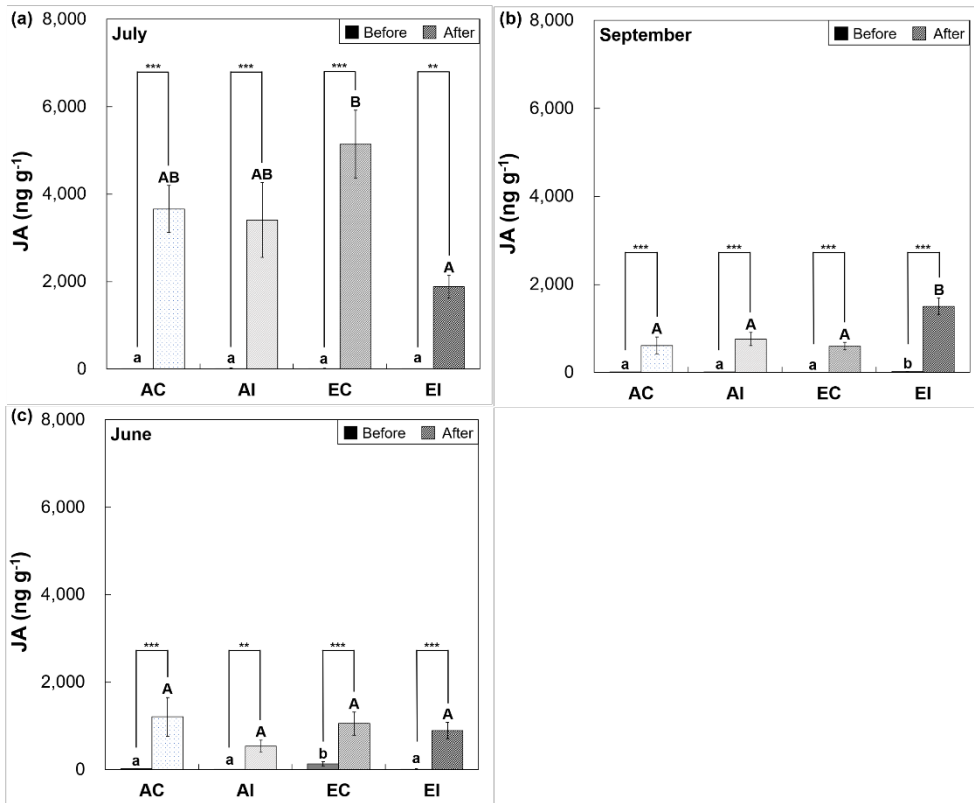


Figure 4-10. Significant increases in jasmonic acid (JA) content were observed after specialist herbivorous attack regardless of seasons, but there were significant differences among experimental groups in July and September. JA content in *A. contorta* leaves before and after OS treatment of July (a), September (b), and June (c). The vertical bars show the standard error for each group (*n* = 9, 12, 12 for each group in June, July, September, respectively). Different letters indicate statistically different sub-groups by Duncan's post-hoc test (*p* < 0.05). Asterisks on each line shows significances of differences between seasons according to *t*-test (*: *p* < 0.05; **: *p* < 0.01; *: *p* < 0.001).**

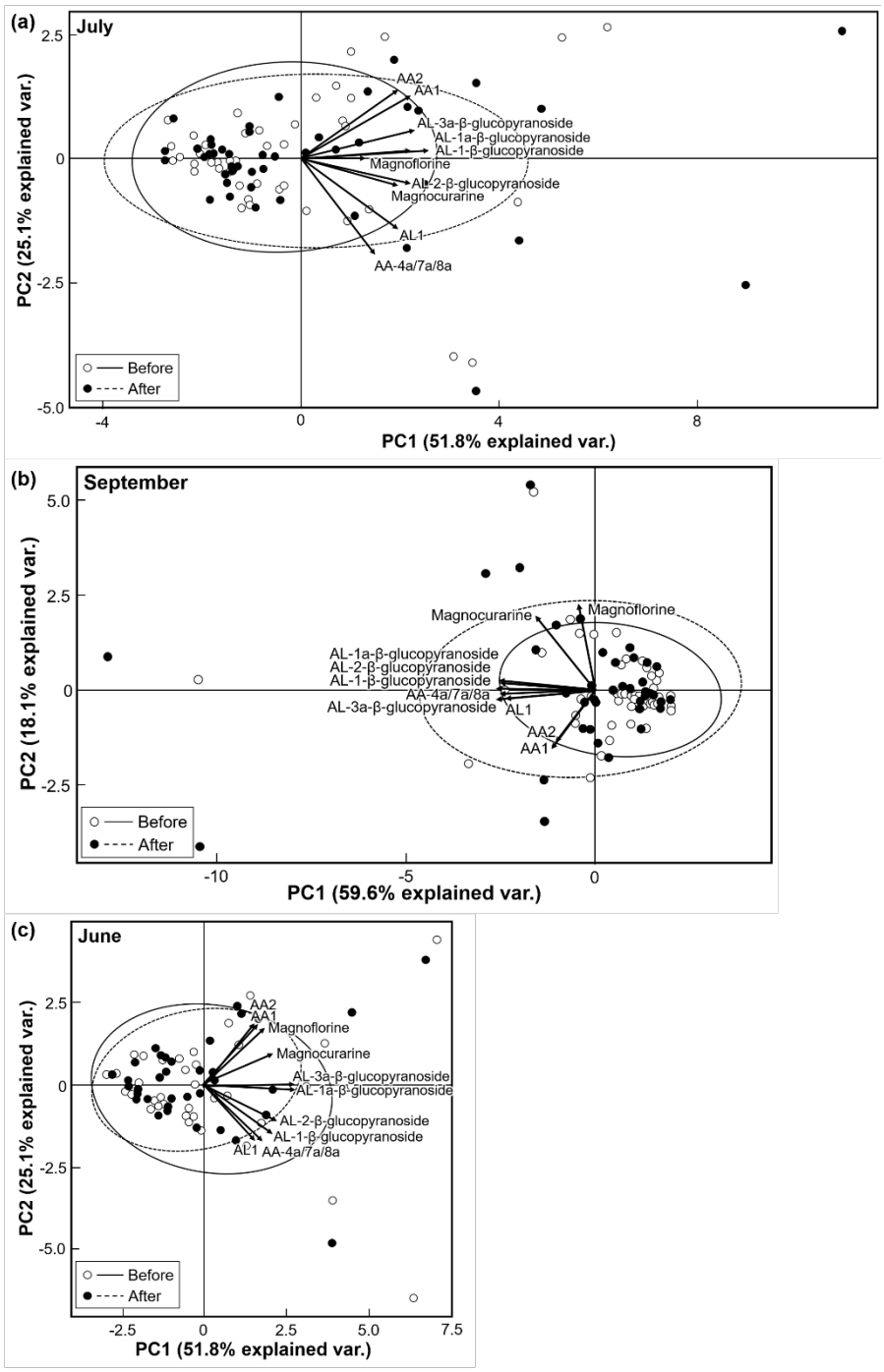


Figure 4-11. No clear differences in secondary metabolites between before and after OS treatment regardless of seasons according to principal component analysis (PCA). PCA results of July (a), September (b), and June (c).

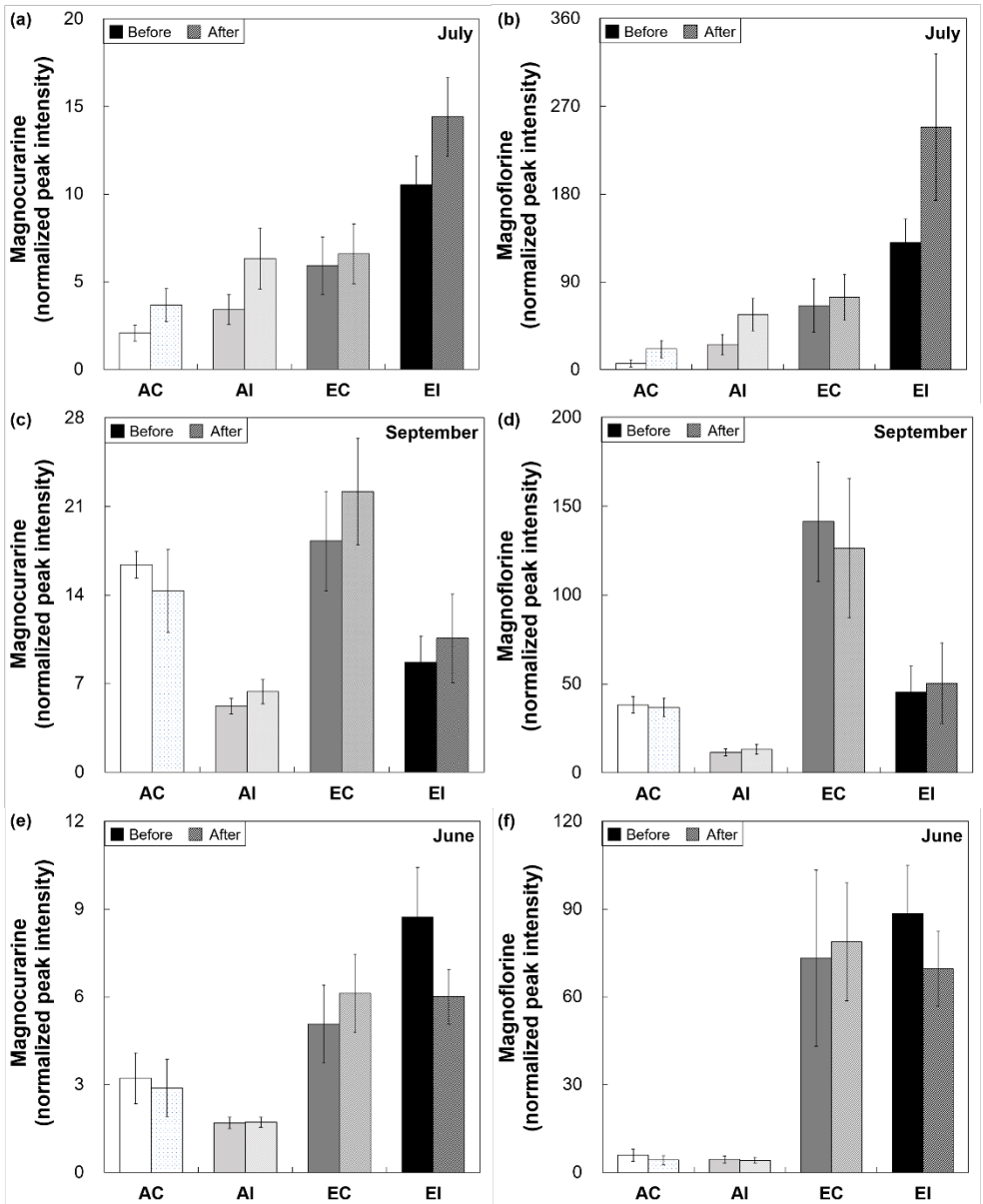


Figure 4-12. Magnocurarine and magnoflorine increased after specialist herbivore attack in July, although there was no statistical significance. Magnocurarine (a, c, e) and magnoflorine (b, d, f) content in *A. contorta* leaves before and after OS treatment of July, September, and June (a, b: July; c, d: September; e, f: June). The vertical bars show the standard error for each group (*n* = 9, 12, 12 in June, July, and September, respectively).

4.4. Discussion

4.4.1. The seasonality of nutrient value and defenses in plants and relative growth performances of specialist and generalist herbivore

In my observations, relative growth performance of specialist herbivore, *S. montela*, had different patterns temporally (Fig. 4-3a). In this study, examinations of herbivore growth performance and host plant properties were made for three times with the consideration of emergence timing of *S. montela* in nature (Shin, 1974). Based on this design, I tried to focus on the possibility of extrapolation for natural life cycle and interactions, not the exploration for temporal variations in detail. Under the present environment, which could be represented as AC of this study, the growth of *S. montela* decreased in the late plant growing season (September), but that increased again in the early plant growing season (June) (Fig. 4-3a). This seems to have a close relationship with nutrient value and constitutive defenses of host plant. C: N ratio, commonly considered as a typical parameter for nutrient value, obviously increased in September and did not significantly change in June for all experimental groups including AC (Fig. 4-4). Besides C: N ratio, further detail information about nutrient value could be assessed by the primary metabolites, particularly nutritious compounds (Royer et al., 2013). In this study, the changes in soluble sugar contents of *A. contorta* in AC in September did not have any statistical significances, whereas free amino acid contents had apparently decreased (Fig. 4-6). Herbivores gain the nutrients for survival and growth such as sugars and proteins from their host

plants (Felton, 1996), thereby the contents of those substances are important for herbivores (de Bruyn et al., 2002). Most of all, although there might be unexpected effects of a single chamber for replicates of experimental plants, the changes which appeared in this study are thought to be reliable because of consistence with well-known knowledges in plant physiology. The contents of these nutritious primary metabolites can be relatively large in the middle of plant growing season, because photosynthesis activity of plants is the highest at that time (Palacio et al., 2007; Gago et al., 2016). On the other hand, in the late growth period, not only low photosynthesis rate but also resource allocation from sources to sinks could cause less amount of sugars and amino acids in developed leaves compared to the middle of growth period (Madsen, 1991; Iwasa, 2000). Thus, the changes in nutrient value of *A. contorta* are likely to have the seasonality of both improvement and decline according to the stage of growth period, like shown in AC of this study.

In contrast to nutrient value, plant constitutive defenses in AC appeared the largest and the smallest in the late and early plant growing season, respectively, shown as the variations in total amount of defensive secondary metabolites (Fig. 4-8). Particularly, the increases in secondary metabolites were remarkable in the late plant growing season (Fig. 4-8), including magnocurarine and magnoflorine (Fig. 4-9a, b), which are thought to be more influential to *S. montela* than aristolochic acids (McKenzie and Price, 1953; Tahir, 1991). Most plants store their secondary metabolites including nitrogen compounds for defensive mechanisms (Bennett and Wallsgrove, 1994; Makkar et al., 2007). Although specialist herbivores have specific adaptive mechanisms for certain toxic metabolites of their host plant, increases in

those compounds could be stressful for them (Ali and Agrawal, 2012). Indeed, *S. montela* can reduce the toxicity of several defensive metabolites of *A. contorta* including aristolochic acids (Nishida, 2002). Nonetheless, strengthened constitutive defenses of *A. contorta* caused by large increases in secondary metabolites would impede the relative growth performance of specialist herbivore in the late of plant growing seasons (Fig. 4-3a). In addition, impeding effects on herbivore growth caused by slightly more secondary metabolites of *A. contorta* could appear in the middle of plant growing season, resulting offsetting the positive effects of improved nutrient value in July (Fig. 4-3a). Thereby, growth performance of June would also be similar with July (Fig. 4-3a), because of the interactive effects of low nutrient value and little constitutive defense resulted from small contents of primary and secondary metabolites.

In the case of induced defensive responses of *A. contorta*, it showed different tendency from nutrient value and constituent defenses. Interestingly, although JA was increased by OS treatment substantially (Fig. 4-10), but secondary metabolites did not show remarkable variations (Fig. 4-11). Because of adaptation of specialist herbivore, it would be ineffective for plants to invest the resources to enlarge the amount of their own defensive metabolites as responses to specialist herbivores (Agrawal, 2000; Zangerl, 2003). Hence, host plants often use other defensive strategies when they recognize the elicitor from specialist herbivore, such as synthesizing other types of metabolites (Agrawal, 2007; Mithöfer and Boland, 2012). Considering lower relative growth performance of *S. montela* when OS-induced leaves provided (Fig. 4-3b), there might be other inducible defense

mechanisms in *A. contorta* for their specialist herbivore which cannot be observed in secondary metabolites, such as volatiles emission or primary metabolites allocation (Kessler and Baldwin, 2001; Steinbrenner et al., 2011). Thus, it is thought that further examination of inducible defensive responses of *A. contorta* to *S. montela* would be necessary.

Contrary to specialist herbivore, the relative growth performance of generalist herbivore (*S. exigua* in this study) was vigorous only in the early plant growing season (Fig. 4-3c). Even though generalist herbivore can utilize various types of plants including highly toxic plant like *A. contorta*, their survival rate rises when they can access less toxic plant simultaneously (Bernays et al., 1994). In addition, they cannot survive well under larger toxic compounds which are over their tolerant threshold (Harvey et al., 2005; Kos et al., 2012). That is, *S. exigua* did not seem to endure continuous exposure to high content of defensive secondary metabolites of *A. contorta*, so that they could survive and grow when toxic metabolites of the plant were the least in June.

4.4.2. Effects of elevated CO_2 and increased watering frequency on the seasonality of host plant and specialist and generalist herbivore

In this study, elevated CO_2 and increased watering frequency affected relative growth performance of specialist herbivore in different ways. Most of all, relative growth performance of all experimental groups decreased in September, and slightly increased in June (Fig. 4-3a). Among experimental

groups, the seasonal variations in relative growth performance of *S. montela* only in EI was similar with that in AC (Fig. 4-3a). Relative growth performance of *S. montela* in other two groups (AI and EC) were the highest and lowest, respectively (Fig. 4-3a). It is likely that the different dynamics of nutrient value and constitutive defenses caused by increased watering frequency are responsible for these distinct herbivore growth patterns from AC. Under increased watering frequency, free amino acids were the largest in July, whereas those were decreased in September like AC (Fig. 4-6). However, unlike AC, contents of soluble sugars were relatively large in AI and EI than in July (Fig. 4-6a, Table 4-1). Since the appropriate ratio of sugars and amino acids is more important rather than exact amount as the nutrient value for herbivore (Machado et al., 2015), more proper nutritious balance with increased soluble sugars in two increased watering frequency seemed to lead to larger herbivore growth. Plants modify total soluble sugar content into higher content under both of water deficit and sufficient condition than normal state according to osmosis (Evans and Reid, 1988). That is, plants tend to maintain larger amount of soluble sugars to make stronger water potential enable to uptake more water (Irigoyen et al., 1992; Lee et al., 2008). In other ways, plants also have to hold more soluble sugars in leaf to retain appropriate osmotic concentration, preventing any possible troubles in osmotic homeostasis under excessive soil water condition (Evans and Reid, 1988). In this study, excessive soil water availability caused by increased watering frequency seemed to be much more influential factor rather than possible dry condition under control watering frequency, because *A. contorta* inhabits the locations where they can easily be exposed to dry condition

(Park et al., 2019; Park et al., 2020). Based on these facts, increased watering frequency seemed to make the nutrient value of *A. contorta* leaves more suitable for its specialist herbivore in the middle of growing season, which could have positive effects on herbivore growth. However, both AI and EI had positive effects of nutrient value, but the relative growth performance was lower in EI than AI (Fig. 4-3a). That is, there would be the effect of different magnitude of constitutive defenses caused by elevated CO_2. In this study, *A. contorta* in EI as well as EC had more secondary metabolites than AI and AC in July, indicating stronger constitutive defenses (Fig. 4-8). Indeed, plants under elevated CO_2 could synthesize more defensive compounds (Zavala et al., 2013; Xu et al., 2019), possibly leading to enhanced defensive mechanisms. As a result of that, relatively impeded growth performance of *S. montela* seemed to be observed in EC and EI than AC and AI, respectively.

Contrastingly, in September, the negative effects of elevated CO_2 on specialist herbivore continued as the secondary metabolites accumulated, but increased watering frequency alleviated that negative effects of high CO_2. Most of all, overall relative growth performance of *S. montela* decreased, and particularly the lowest relative growth performance of specialist herbivore was obtained in EC (Fig. 4-3a). This overall decreased growth performance of *S. montela* was likely to be influenced by both decreased nutrient value and increased constitutive defenses in September, shown as higher value of C: N ratio and secondary metabolites content in all experimental groups in September seemed to lead the growth of specialist herbivore impeded (Fig. 4-4, 4-8). These variations of nutrient value are common in perennial plants, which need to prepare next year and relocate essential nutrients into

belowground parts mostly (Pugliese, 1988). As a consequence of lower primary metabolites content, other secondary metabolites could appear in relatively higher content. However, considering similar content of soluble sugars and free amino acids among experimental groups in this study (Fig. 4-6), distinct secondary metabolites amount was thought to be the governing factor for growth pattern of specialist herbivore in September (Fig. 4-8). The highest and second highest total amount of secondary metabolites were observed in EC and EI, respectively (Fig. 4-8). In particular, the most abundant metabolites were magnocurarine and magnoflorine (Fig. 4-9), expected to be more stressful to *S. montela* rather than aristolochic acids they adapted to. That is, constitutive defenses were enlarged by elevated CO_2, which caused larger decrease in relative growth performance of *S. montela*. Plants' acclimation to high CO_2 is species-specific, which can be shown as both of stimulation and inhibition of photosynthesis (Bowes, 1991; Gutschick, 2007). In fact, *A. contorta* shows impeded photosynthesis and consequent decreased growth under elevated CO_2 condition (Fig. 3-10, 3-12). Plant defenses could be intensified with the troubles in growth performance, according to growth-defense trade-off hypothesis (Huang et al., 2019). Therefore, elevated CO_2 could have negative effects on specialist herbivore with these stimulated constitutive defenses in host plants.

In the case of generalist herbivore, elevated CO_2 and increased watering frequency exhibited opposite effects with specialist herbivore (Fig. 4-3c). Unlike specialist herbivore, generalist herbivore showed more active growth under elevated CO_2 condition in June, and the highest value of RGR of *S. exigua* was obtained in EC (Fig. 4-3c). Since they are generalist herbivore,

they could be tolerant to various toxic compounds of plant, including those *A. contorta* contains (Feng et al., 2012; Jeude and Fordyce, 2014). In particular, when secondary metabolites are not yet gathered as much as in the early of growing season, their growth performance might be rather closely related to primary metabolites (Bernays, 1998; Lemoine et al., 2013). Although the differences in primary metabolites were not clear, free amino acids in EC were slightly larger than other groups in June (Fig. 4-6b), possibly indicating better nutritious condition for herbivore. Elevated CO_2 can have both positive and negative effects on plant growth and photosynthesis according to species characteristics (Bowes, 1991), but weak stimulating effects could be observed initially because of carbon fertilization by elevated CO_2 (DeLucia et al., 1985). Therefore, it could be suggested that there may be little competitive pressure on *S. montela* related to food resources in the early growing seasons of plant restrictively, because of the accessibility of generalist herbivore. In addition, that pressure might be more intensified under climate change especially. Nonetheless, as generalist herbivore could not grow with accumulated secondary metabolites, the competitive stress would hardly exist except the early plant growing seasons, regardless of CO_2 levels and watering frequencies.

Although the variations in larval weights were only examined in this study, future changes in population development of *S. montela* under climate change could be conjectured considering the contribution of larval weights to population development of insects. In this study, elevated CO_2 reduced larval weights by enhancement of constitutive defenses in host plant (Fig. 4-8, 9) and possibly increased competitive stress from generalist herbivore by weak

improvement of nutrient value in the early plant growing season (Fig. 4-3c, 6). Several values such as the success rate of adult emergence from pupae, the rate of mating, and the fecundity of female adults are commonly considered to estimate population development of insects (Birch, 1948; Howe, 1953). Those values generally have positive correlations with the larval size (Harvey and Corbet, 1985; Weaver and McFarlane, 1990; Hirschberger, 1999). That is, large increase of population size could be expected from high weights of larvae. Considering these facts, elevated CO_2 seemed to have negative effects on the population development of *S. montela*, but slight compensation of those negative effects of CO_2 could occur by increased watering frequency. Indeed, under EI condition of this study representing climate change environment, the growth performance of *S. montela* was similar with AC condition which reflects the present environment (Fig. 4-3a). Thus, weaker threats of climate change on some specialist herbivores might appear because of the interactions between various changes in environmental factors, particularly elevated CO_2 and increased precipitation frequency.

4.5. Conclusion

Relative growth performance of specialist and generalist herbivore had different seasonality, according to the variations in plant primary and secondary metabolites. Interestingly, primary and secondary metabolites of host plant showed distinct variations according to seasons, which seemed to be responsible for the seasonal dynamics of those herbivores (Fig. 4-13). Generalist herbivore could survive in the early growing season of host plant, but they could not survive after the middle of growing season because of accumulation of defensive secondary metabolites. In contrast, growth performance of specialist herbivore would be the lowest in the late plant growing season by impeded nutrient value and largely enhanced constitutive defenses, whereas it could be similar in the early and late plant growing season by the offsets of nutrient value and plant constitutive defenses. Additionally, two environmental factors of climate change, elevated CO_2 and increased watering frequency, affected in different ways temporally. Increased watering frequency showed positive effects on nutrient value improvement based on nutritious balance in the middle of growth period, and another positive effects by reducing constitutive defense in the late growth period. Contrariwise, elevated CO_2 had cumulative effects and it always acted negatively with enhanced constitutive defenses, possibly in accordance with growth-defense trade-off hypothesis. Therefore, the development of *S. montela* would have different influential factors according to the phase of plant growth period under climate change. That is, they might be slightly threatened by competition in the early growth period, and could

be impeded by largely stimulated constitutive defense of host plant. However, based on more improved nutrient value than increased constitutive defenses of host plant, their population development could be compensated in the middle of plant growth period, resulting less risk as climate change progress. The findings of this study suggest that consideration of seasonality in plant primary and secondary metabolites is important not only for detail understating for plant-herbivore interactions, but also for more precise and sophisticated predictions for indirect effects of climate change on plant-insect interactions.

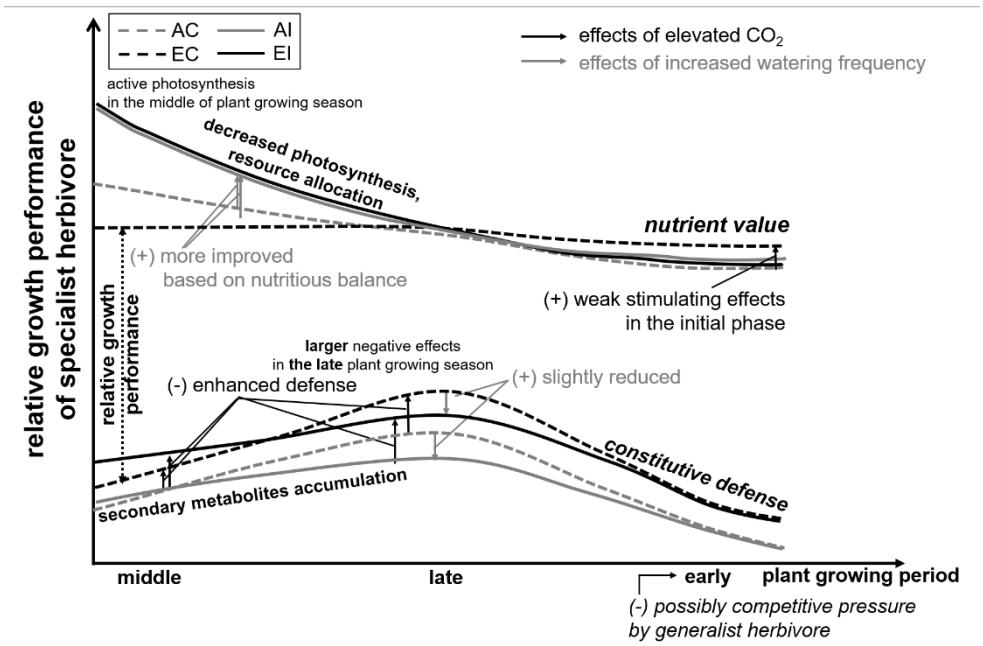


Figure 4-13. A schematic diagram representing the seasonality of plant physiological traits and relative growth performance of specialist herbivore, including temporal variations of effects of elevated CO_2 and increased watering frequency.

Chapter 5. General conclusion

There is no doubt that climate change affects ecosystems in various level, and future biodiversity will have substantial variations under rapid environmental changes. It is required to figure out the effects of those environmental changes on plant-herbivore interaction, particularly for specialist herbivore, based on their ecological and biological values in ecosystem. Although there are large number of researches to estimate the effects of climate change on plant-herbivore interaction, it is still necessary to consider other possibly variable abiotic factors because of the complexity of climate change, and also evaluate the effects of climate change on plant-herbivore interaction comprehensively under the consideration of whole life cycle of host plant and its herbivore.

Therefore, I conducted three major researches: a field survey and two mesocosm experiments using OTC for a native host plant (*A. contorta*) and its specialist herbivore (*S. montela*) (Fig. 5-1). First, I found possible effects of various environmental factors on ecological traits of *A. contorta* population. Both biotic and abiotic factors can vary the growth period of *A. contorta* by advancing the flowering period. In particular, herbivorous pressure, interspecific competitive stress, and cation contents are considered as significantly potential environmental factors for the growth and development of *A. contorta*.

In addition, I also found individual and/or interactive effects of CO_2 elevations and soil water content, highly predictable to occur under climate change, on the interaction between *A. contorta* and *S. montela*. Interestingly,

unlike recent major prediction, the elevated CO_2 decreased growth of host plant by impeding photosynthesis. High CO_2 also increased the contents of secondary metabolites in plant leaves, causing negative consequences on growth performance of its specialist herbivore. Increased precipitation frequency, however, appeared to have different effects on plant growth and defenses according to CO_2 concentration. In the perspective of plant growth, it played roles as both constraint and ameliorator under ambient and elevated CO_2, respectively, but in the perspective of plant defenses, it alleviated constitutive defenses regardless of CO_2 level. Nonetheless, because of significant CO_2 effects, this study suggests that both the quantity and quality of host plants would decline, and the growth performance of its specialist herbivore might be threatened as climate change progresses. That is, although the detail scenario differed, decline of insect community is likely to be caused by climate change, consistent with the recent estimates.

Furthermore, I first observed these effects of CO_2 and soil water content had different seasonality even. In particular, the seasonality of climate change effects was highly related to different seasonal dynamics of nutrient value and constitutive defenses of host plant. Increased precipitation frequency showed strong positive effects on nutrient value and constitutive defenses of host plant especially in the middle of plant growing season, regardless of CO_2 level. Contrary to these remarkable positive effects in the middle of plant growing season, it did not have significant effects compared to elevated CO_2 in the late of plant growing season. Elevated CO_2 had cumulative negative effects on both nutrient value and constitutive defenses, that is, more significant effects were observed in the late of plant growing

season. As complex consequences of the effects of these two environmental factors, the growth performance of specialist and generalist herbivore showed clearly distinct seasonal variations. Thereby, it could be suggested that effects of climate change on plant-herbivore interaction would have considerable seasonal dynamics with different influential factors. Moreover, it could also be insisted that less danger of herbivorous insect by the ameliorating effect of increased precipitation frequency to high CO_2 at a certain emergence timing in their life cycle.

Additionally, the findings of this research provided detail experimental evidences for the effects of variable environmental factors under climate change on plant and specialist herbivore interaction, being aware of the possibility of domination by species-specific characteristics. This study also highlighted the importance of increased precipitation frequency as an influential environmental factor in climate change research, and ecological implications of seasonal dynamics to deepen the understanding of future plant-herbivore interaction under climate change.

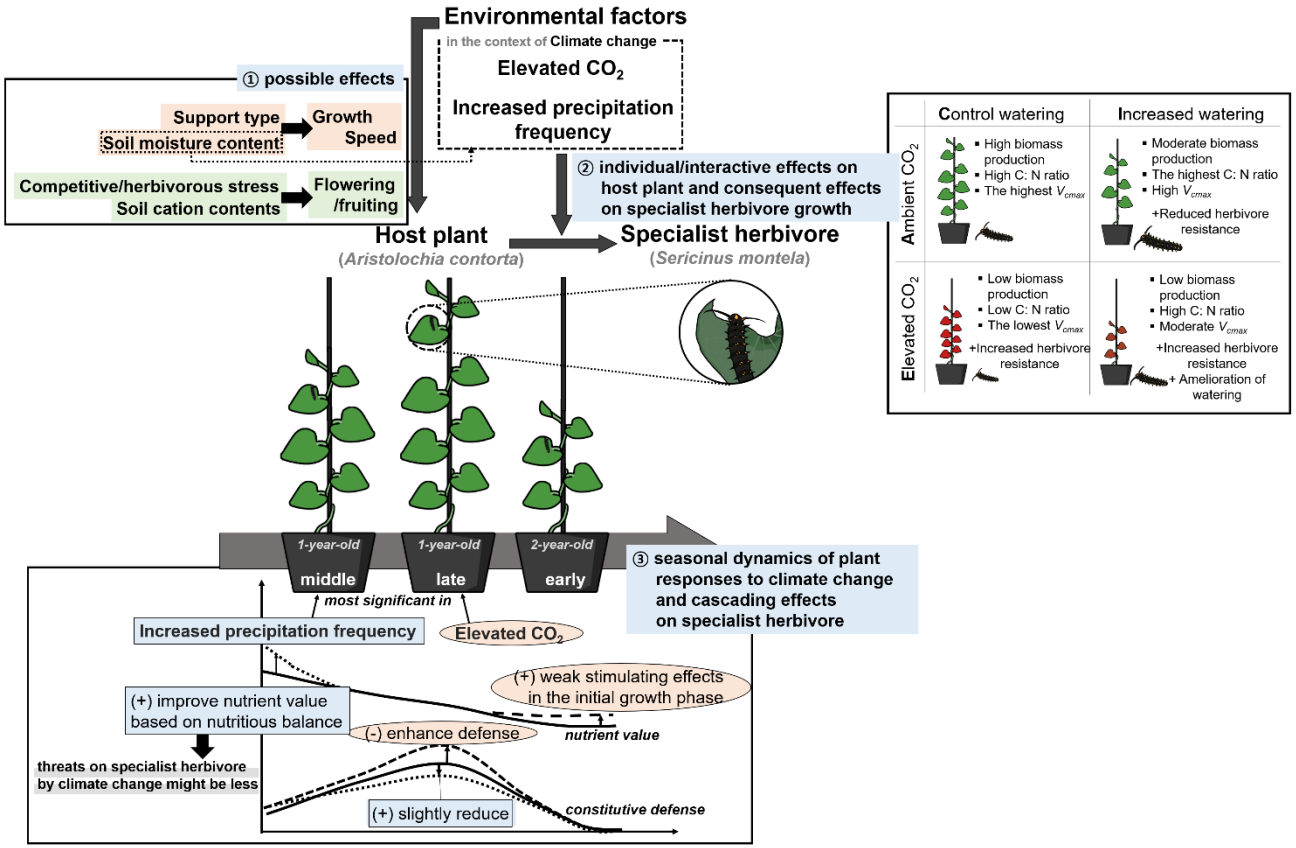


Figure 5-1. A schematic diagram showing the general conclusion of this research

References

Agrawal, A.A., 2000. Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae). *Ecology. 81*, 1804-1813.

Agrawal, A.A., 2007. Macroevolution of plant defense strategies. *Trends. Ecol. Evol. 22*, 103-109.

Agrawal, A.A., Fishbein, M., 2006. Plant defense syndromes. *Ecology. 87*, S132-S149.

Ainsworth, E.A., Rogers, A., 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant. Cell. Environ. 30*, 258-270.

Akula, R., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant. Signal. Behav. 6*, 1720-1731.

Albert, K.R., Mikkelsen, T.N., Michelsen, A., Ro-Poulsen, H., van der Linden, L., 2011. Interactive effects of drought, elevated CO_2 and warming on photosynthetic capacity and photosystem performance in temperate heath plants. *J. Plant. Physiol. 168*, 1550-1561.

Ali, J.G., Agrawal, A.A., 2012. Specialist versus generalist insect herbivores and plant defense. *Trends. Plant. Sci. 17*, 293-302.

Allen, S.E., Grimshaw, H.M., Parkinson, J.A., Quarmby, C., 1974. *Chemical analysis of ecological materials*, Blackwell Scientific Publications, Oxford, UK.

Alnsour, M., Ludwig-Müller, J., 2015. Potential effects of climate change on plant primary and secondary metabolism and its influence on plant ecological interactions. *J. Endocyt. and Cell. Res. 26*, 90-99.

Anulika, N.P., Ignatius, E.O., Raymond, E.S., Osasere, O-I., Abiola, A.H., 2016. The chemistry of natural product: plant secondary metabolites. Int. J. Technol. Enhanc. Emer. Eng. Res. 4, 1-8.

Arp, W.J., 1991. Effects of source-sink relations on photosynthetic acclimation to

elevated CO_2. *Plant. Cell. Environ. 14*, 869-875.

Aucott, M., 2019. Are changes in plants due to enhanced CO_2 contributing to insect population declines? *Environ. Entomol. 48*, 274-275.

Austin, A.T., Ballaré, C.L., 2014. Plant interactions with other organisms: molecules, ecology and evolution. *New. Phytol. 204*, 257-260.

Baldwin, I.T., 2001. An ecologically motivated analysis of plant-herbivore interactions in native tobacco. *Plant. Physiol. 127*, 1449-1458.

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J., Good, J.E.G., Harrington, R., Hartley, S., Jones, T.H., Lindroth, R.L., Press, M.C., Symrnioudis, I., Watt, A.D., Whittaker, J.B., 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Glob. Change. Biol. 8*, 1-16.

Basset, Y., Lamarre, G.P.A., 2019. Toward a world that values insects. *Science. 364*, 1230-1231.

Becklin, K.M., Anderson, J.T., Gerhart, L.M., Wadgymar, S.M., Wessinger, C.A., Ward, J.K., 2016. Examining plant physiological responses to climate change through an evolutionary lens. *Plant. Physiol. 172*, 635-649.

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., Courchamp, F., 2012. Impacts of climate change on the future of biodiversity. *Ecol. Lett. 15*, 365-377.

Bengtsson, B.O., Ceplitis, A., 2000. The balance between sexual and asexual production in plants living in variable environments. *J. Evol. Biol. 13*, 415-422.

Bennett, R.N., Wallsgrove, R.M., 1994. Secondary metabolites in plant defence mechanisms. *New. Phytol. 127*, 617-633.

Berenbaum, M.R., Zangerl, A.R., Lee, K., 1989. Chemical barriers to adaptation by a specialist herbivore. *Oecologia, 80*, 501-506.

Bernays, E.A., 1998. Evolution of feeding behavior in insect herbivores. *Bioscience. 48*, 35-44.

Bernays, E.A., Bright, K.L., Gonzalez, N., Angel, J., 1994. Dietary mixing in a generalist herbivore: tests of two hypotheses. *Ecology. 75*, 1997-2006.

Bichsel, R.G., Starman, T.W., Wang, Y., 2008. Nitrogen, phosphorus, and potassium requirements for optimizing growth and flowering of the nobile dendrobium as a potted orchid. *Hortscience. 43*, 328-332.

Birch, L.C., 1948. The intrinsic rate of natural increase of an insect population. *J. Anim. Ecol. 17*, 15-26.

Bowes, G., 1991. Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. *Plant. Cell. Environ. 14*, 795-806.

Boyle, J., 2004. A comparison of two methods for estimating the organic matter content of sediments, *J. Paleolimnol. 31*, 125–127.

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil. Sci. 59*, 39–46.

Brody, A.K., 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. *Ecology. 78*, 1624-1631.

Broughton, K.J., Smith, R.A., Duursma, R.A., Tan, D.K.Y., Payton, P., Bange, M.P., Tissue, D.T., 2017. Warming alters the positive impact of elevated CO_2 concentration on cotton growth and physiology during soil water deficit. *Func. Plant. Biol. 44*, 267-278.

de Bruyn, L., Scheirs, J., Verhagen, R., 2002. Nutrient stress, host plant quality and herbivore performance of a leaf-mining fly on grass. *Oecologia, 130*, 594-599.

Buse, A., Good, J.E.G., 1996. Synchronization of larval emergence in winter moth (*Operophtera brumata* L.) and budburst in pedunculate oak (*Quercus robur* L.) under simulated climate change. *Ecol. Entomol. 21*, 335-343.

CaraDonna, P.J., Iler, A.M., Inouye, D.W., 2014. Shifts in flowering phenology reshape a subalpine plant community. *PNAS. 111*, 4916-4921.

Cha, D., Lee, D., Jin, C., Kim, G., Choi, Y., Suh, M., Ahn, J., Hong, S., Min, S., Park, S., Kang, H., 2016. Future changes in summer precipitation in regional climate simulations over the Korean Peninsula forced by multi-RCP scenarios of HadGEM2-AO. *Asia-Pac. J. Atmos. Sci. 52*, 139-149.

Chapin, F.S., Bloom, A.J., Field, C.B., Waring, R.H., 1987. Plant responses to multiple environmental factors. *Bioscience. 37*, 49-57.

Chen, M.-S., 2008. Inducible direct plant defense against insect herbivores: a review. *Insect. Sci. 15*, 101-114.

Cheung, T.P., Xue, C., Leung, K., Chan, K., Li, C.G., 2006. Aristolochic acids detected in some raw Chinese medicinal herbs and manufactured herbal products–a consequence of inappropriate nomenclature and imprecise labelling? *Clin. Toxicol. 44*, 371-378

Cho, L.H., Yoon, J., An, G., 2016. The control of flowering time by environmental factors. *Plant. J. 90*, 708-719.

Choi, S.W., Kim, S.S., 2011. The past and current status of endangered butterflies in Korea. *Entomol. Sci. 15*, 1-12.

Clayton, D.H., Bush, S.E., Johnson, K.P., 2015. *Coevolution of life on hosts: integrating ecology and history*. The University of Chicago Press, Chicago, USA.

Coley, P.D., 1998. Possible effects of climate change on plant/herbivore interactions in moist tropical forests. *Clim. Change. 39*, 455-472.

Cook, R.E., 1979. Asexual reproduction: a further consideration. *Am. Nat. 113*, 769-772.

Cornell, H.V., Hawkins, B.A., 2003. Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. *Am. Nat. 161*, 507-

522.

Delucia, E.H., Nabity, P.D., Zavala, J.A., Berenbaum, M.R., 2012. Climate change: resetting plant-insect interactions. *Plant. Physiol. 160*, 1677-1685.

DeLucia, E.H., Sasek, T.W., Strain, B.R., 1985. Photosynthetic inhibition after long-term exposure to elevated levels of atmospheric carbon dioxide. *Photosynth. Res. 7*, 175-184.

Donoso, I., Stefanescu, C., Martinez-Abrain, A., Traveset, A., 2016. Phenological asynchrony in plant-butterfly interactions associated with climate: a community-wide perspective. *Oikos. 125*, 1434-1444.

Dore, M.H.I., 2005. Climate change and changes in global precipitation patterns: What do we know? *Environ. Int. 31*, 1167-1181.

Dorji, T., Hopping, K.A., Meng, F., Wang, S., Jiang, L., Klein, J.A., 2020. Impacts of climate change on flowering phenology and production in alpine plants: the importance of end of flowering. *Agr. Ecosyst. Environ. 291*, 106795.

Drake, B.G., González-Meler, M.A., Long, S.P., 1997. More efficient plants: a consequence of rising atmospheric CO_2 concentration? *Annu. Rev. Plant. Phys. 48*, 609-639.

Elzinga, J.A., Atlan, A., Biere, A., Gigord, L., Weis, A.E., Bernasconi, G., 2007. Time after time: flowering phenology and biotic interactions. *Trends. Ecol. Evol. 22*, 432-439.

Evans, R.Y., Reid, M.S., 1988. Changes in carbohydrates and osmotic potential. *J. Amer. Soc. Hort. Sci. 113*, 884-888.

Feng, X., Jiang, H., Zhang, Y., He, W., Zhang, L., 2012. Insecticidal activities of ethanol extracts from thirty Chinese medicinal plants against *Spodoptera exigua* (Lepidoptera: Noctuidae). *J. Med. Plants. Res. 6*, 1263-1267.

Feeny, P., 1976. Plant apparency and chemical defense. In: Wallace, J.W., Mansell, R.L., (eds) *Biochemical Interaction Between Plants and Insects*. Springer,

Boston, USA. pp. 1-40.

Felton, G.W., 1996. Nutritive quality of plant protein: sources of variation and insect herbivore responses. *Arch. Insect. Biochm. Physiol. 32*, 107-130.

Fowler, M.D., Kooperman, G.J., Randerson, J.T., Pritchard, M.S., 2019. The effect of plant physiological responses to rising CO_2 on global streamflow. *Nat. Clim. Change. 9*, 873-879.

Freeland, W.J., 1991. Plant secondary metabolites: biochemical coevolution with herbivores. In: *Plant Defenses Against Mammalian Herbivory* (eds) Palo, R.T., and Robbins, C.T., CRC Press, USA.

Futuyma, D.J., Agrawal, A.A., 2009. Macroevolution and the biological diversity of plants and herbivores. *PNAS. 106*, 18054-18061.

Gago, J., Daloso, D.M., Figueroa, C.M., Flexas, J., Fernie, A.R., Nikoloski, Z., 2016. Relationships of leaf net photosynthesis, stomatal conductance, and mesophyll conductance to primary metabolism: a multispecies meta-analysis approach. *Plant. Physiol. 171*, 265-279.

Ge, Z.M., Zhou, X., Kellomäki, S., Peltola, H., Martikainen, P.J., Wang K.Y., 2012. Acclimation of photosynthesis in a boreal grass (*Phalaris arundinacea* L.) under different temperature, CO_2, and soil water regimes. *Photosynthetica. 50*, 141-151.

Ghosh, P., Pal, P., 2010. Effect of different sources and levels of potassium on growth, flowering and yield of African marigold (*Tagetes erecta* Linn.) cv. 'Siracole'. *Indian. J. Nat. Prod. Resour. 1*, 371-375.

Gianoli, E., 2003. Phenotypic responses of the twining vine *Ipomoea purpurea* (Convolvulaceae) to physical support availability in sun and shade. *Plant. Ecol. 165*, 21-26.

Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W., Holt, R.D., 2010. A framework for community interactions under climate change. *Trends. Ecol.*

Evol. 25, 325-331.

Gong, B., Zhang, G., 2014. Interactions between plants and herbivores: a review of plant defense. *Acta. Ecol. Sin. 34*, 325-336.

Goverde, M., Arnone, J.A.III, Erhardt, A., 2002. Species-specific reactions to elevated CO_2 and nutrient availability in four grass species. *Basic. Appl. Ecol. 3*, 221-227.

Gutschick, V.P., 2007. Plant acclimation to elevated CO_2 - from simple regularities to biogeographic chaos. *Ecol. Model. 200*, 433-451.

Hamerlynck, E.P., Huxman, T.E., Loik, M.E., Smith, S.D., 2000. Effects of extreme high temperature, drought and elevated CO_2 on photosynthesis of the Mojave Desert evergreen shrub, *Larrea tridentate*. *Plant. Ecol. 148*, 183-193.

Hartmann, T., 2004. Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. *Planta. 219*, 1-4.

Hartmann, T., 2007. From waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phytochemistry, 68*, 22-24.

Harvey, I.F., Corber, P.S., 1985. Territorial behavior of larvae enhances mating success of male dragonflies. *Anim. Behav. 33*, 561-565.

Harvey, J.A., van Nouhuys, S., Biere, A., 2005. Effects of quantitative variation in allelochemicals in *Plantago lanceolata* on development of a generalist and a specialist herbivore and their endoparasitoids. *J. Chem. Ecol. 31*, 287-302.

ul Hassan, M.N., Zainal, Z., Ismail, I., 2015. Green leaf volatiles: biosynthesis, biological functions and their applications in biotechnology. *Plant. Biotech. J. 13*, 727-739.

Hirschberger, P., 1999. Larval population density affects female weight and fecundity in the dung beetle *Aphodius ater*. *Ecol. Entomol. 24*, 316-322.

Howe, R.W., 1953. The rapid determination of the intrinsic rate of increase of an insect population. *Annal. Appl. Biol. 40*, 134-151.

Huang, J., Hammerbacher, A., Weinhold, A., Reichelt, M., Gleixner, G., Behrendt, T., van Dam, N.M., Sala, A., Gerschenzon, J., Trumbore, S., Hartmann, H., 2019. Eyes on the future – evidence for trade-offs between growth, storage and defense in Norway spruce. *New. Phytol. 222*, 144-158.

IPCC, 2001. Climate Change 2001: The scientific basis. In: *Contribution of Working Group I to the Third Assessment Report of the Inter-governmental Panel on Climate Change*. Intergovernmental Panel on Climate Change, Geneva.

IPCC. 2007. Climate Change 2007: The physical science basis. In: *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.

IPCC. 2014. Climate change 2014 synthesis report. In: *Contribution of Working Group I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.

Irigoyen, J.J, Einerich, D.W., Sanchez-Diaz, M., 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativd*) plants. *Physiol. Plantarum. 84*, 55-60.

Ivie, G.W., Bull, D.L., Beier, R.C., Pryor, N.W., Oertli, E.H., 1983. Metabolic detoxification: mechanism of insect resistance to plant psoralens. *Science. 221*, 374-376.

Iwasa, Y., 2000. Dynamic optimization of plant growth. *Evol. Ecol. Res. 2*, 437-455.

Jamieson, M.A., Trowbridge, A.M., Raffa, K.F., Lindroth, R.L., 2012. Consequences of climate warming and altered precipitation patterns for plant-insect and multitrophic interactions. *Plant. Physiol. 160*, 1719-1727.

Je, S.-M., Woo, S.Y., Lee, S.H., Kwak, M.J., Lee, T.Y., Kim, S.H., 2018. Combined effect of elevated CO_2 concentration and drought on the photosynthetic apparatus and leaf morphology traits in seedlings of yellow poplar. *Ecol. Res. 33*, 403-412.

Jeude, S.E., Fordyce, J.A., 2014. The effects of qualitative and quantitative variation of aristolochic acids on preference and performance of a generalist herbivore. *Entomol. Exp. Appl. 150,* 232-239.

Joham, H.E., 1986. Effects of nutrient elements on fruiting efficiency. *Cotton Physiology Book 1*. The Cotton Foundation, Memphis, USA, 79-90.

Johnson, S.N., Hartley, S.E., 2017. Elevated carbon dioxide and warming impact silicon and phenolic-based defences differently in native and exotic grasses. *Glob. Change. Biol. 24*, 3886-3896.

Johnson, S.N., Waterman, J.M., Hall, C.R., 2020. Increased insect herbivore performance under elevated CO_2 is associated with lower plant defence signaling and minimal declines in nutritional quality. *Sci. Rep. 10*, 14553.

Kamphake, L.J., Hannah, S.A., Cohen, J.M., 1967. Automated analysis for nitrate by hydrazine reduction. *Water. Res. 1*, 205–216.

Keddy, P.A., 2017. Two extreme cases of stress tolerance, In: *Plant Ecology: Origins, Processes, Consequences*, 2nd edition. Cambridge University Press, New York, USA. pp. 394-396.

Kessler, A., Baldwin, I.T., 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science. 291*, 2141-2144.

Kimball, B.A., Pinter, Jr.P.J., Wall, G.W., Garcia, R.L., LaMorte, R.L., Jak, P.M.C., Frumau, K.F.A., Vugts, H.F., 1997. Comparisons of responses of vegetation to elevated carbon dioxide in free-air and open-top chamber facilities. *Adv. Carbon. Dioxide. Eff. Res. 61*, 5.

Kingsolver, J.G., Woods, H.A., Buckley, L.B., Potter, K.A., MacLean, H.J., Higgins, J.K., 2011. Complex life cycles and the responses of insects to climate change. *Integr. Comp. Biol. 51*, 719-732.

Klanderud, K., 2008. Species-specific responses of an alpine plant community under simulated environmental change. *J. Veg. Sci. 19*, 363-372.

Knepp, R.G., Hamilton, J.G., Mohan, J.E., Zangerl, A.R., Berenbaum, M.R., DeLucia, E.H., 2005. Elevated CO2 reduces leaf damage by insect herbivores in a forest community. *New. Phytol. 167*, 207-218.

Korea Forest Service, 2016. *National List of Species of Korea*. Korea Forest Service, Seoul.

Korea Meteorological Administration, 2018. *Past climate data*. https://www.weather.go.kr/weather/climate/past_cal.jsp

Kos, M., Houshyani, B., Wietsma, R., Kabouw, P., Vet, L.E.M., van Loon, J.J.A., Dicke, M., 2012. Effects of glucosinolates on a generalist and specialist leaf-chewing herbivore and an associated parasitoid. *Phytochemistry. 77*, 162-170.

Lahive, F., Hadley, P., Daymond, A.J., 2018. The impact of elevated CO_2 and water deficit stress on growth and photosynthesis of juvenile cacao (*Theobroma cacao* L.). *Photosynthetica. 56*, 911-920.

Lee, B.-R., Jin, Y.-L., Jung, W.-J., Avice, J.-C., Morvan-Bertrand, A., Ourry, A., Park, C.-W., Kim, T.-H., 2008. Water-deficit accumulates sugars by starch degradation—not by de novo synthesis—in white clover leaves (*Trifolium repens*). *Physiol. Plantarum. 134*, 403-411.

Lee, G., Joo, Y., Diezel, C., Lee, E.J., Baldwin, I.T., Kim, S-G., 2016. *Trichobaris* weevils distinguish amongst toxic host plants by sensing volatiles that do not affect larval performance. *Mol. Ecol. 25*, 3509-3519.

Lee, H., Kang, W.S., Ahn, M.I., Cho, K., Lee, J.-H., 2016. Predicting temporal shifts in the spring occurrence of overwintered *Scotinophara lurida* (Hemiptera: Pentatomidae) and rice phenology in Korea with climate change. *Int. J. Biometeorol. 60*, 53-61.

Lee, T.B., 2003. *Colored Flora of Korea*, Hyangmunsa, Seoul, Korea.

Lemoine, N.P., Drews, W.A., Burkepile, D.E., Parker, J.D., 2013. Increased

temperature alters feeding behavior of a generalist herbivore. *Oikos. 122*, 1669-1678.

Liancourt, P., Spence, L.A., Song, D.S., Lkhagva, A., Sharkhuu, A., Boldgiv, B., Helliker, B.R., Petraitis, P.S., Casper, B.B., 2013. Plant response to climate change varies with topography interactions with neighbors, and ecotype. *Ecology, 94*, 444-453.

Lincoln, D.E., Fajer, E.D., Johnson, R.H., 1993. Plant-insect herbivore interactions in elevated CO_2 environments. *Trends. Ecol. Evol. 8*, 64-68.

Long, S.P., 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? *Plant. Cell. Environ. 14*, 729-739.

Ma, H.W., Dong, C.Z., Zhou, X.M., Bu, M.M., Yu, S.Q., 2018. Aristololactam derivatives from the fruits of *Aristolochia contorta* Bunge, *Nat. Prod. Res. 32*, 2505-2509.

Machado, R.A.R., Arce, C.C.M., Ferrieri, A.P., Baldwin, I.T., Erb, M., 2015. Jasmonate-dependent depletion of soluble sugars compromises plant resistance to *Manduca sexta*. *New. Phytol. 207*, 91-105.

Madsen, J.D., 1991. Resource allocation at the individual plant level. *Aquat. Bot. 41*, 67-86.

Makkar, H.P.S., Siddhuraju, P., Becker, K., 2007. Plant Secondary Metabolites. Humana Press, USA.

Mao, W.W., Gao, W., Liang, Z.T., Li, P., Zhao, Z.Z., Li, H.J., 2017. Characterization and quantitation of aristolochic acid analogs in different parts of Aristolochiae Fructus, using UHPLCQ/TOF-MS and UHPLC-QqQ-MS. *Chin. J. Nat. Medicines. 15*, 392-400.

Maron, J.L., Agrawal, A.A., Schemske, D.W., 2019. Plant-herbivore coevolution and plant speciation. *Ecology. 100*, e02704.

Mathur, V., Ganta, S., Raaijmakers, C.E., Reddy, A.S., Vet, L.E.M., van Dam, N.M., 2011. Temporal dynamics of herbivore-induced responses in *Brassica juncea* and their effect on generalist and specialist herbivores. *Entomol. Exp. Appl. 139*, 215-225.

Matthews, H.D., 2007. Implications of CO_2 fertilization for future climate change in a coupled climate-carbon model. *Glob. Change. Biol. 13*, 1068-1078.

Mazid, M., Khan, T.A., Mohammad, F., 2011. Role of secondary metabolites in defense mechanisms of plants. *Biol. Med. 3*, 232-249.

McGale, E., Diezel, C., Schuman, M.C., Baldwin, I.T., 2018. Cry1Ac production is costly for native plants attacked by non-Cry1Ac-targeted herbivores in the field. *New. Phytol. 219*, 714-727.

McKenzie, A.W., Price, J.R., 1953. The alkaloids of *Gyrocarpus americanus* Jacq. *Aust. J. Chem. 6*, 180-185.

McKinney, M.L., 1997. Extinction vulnerability and selectivity: combining ecological and paleontological views. *Annu. Rev. Ecol. Syst. 28*, 495-516.

McLeod, A.R., Long, S.P., 1999. Free-air carbon dioxide enrichment (FACE) in global change research: a review. *Adv. Ecol. Res. 28*, 1-56.

van der Meijden, E., 1996. Plant defence, an evolutionary dilemma: contrasting effects of (specialist and generalist) herbivores and natural enemies. In: *Proceedings of the 9th International Symposium on Insect-Plant Relationships*. Springer, USA. pp. 307-310.

Messerli, J., Bertrand, A., Bourassa, J., Bélanger, G., Castonguay, Y., Tremblay, G., Baron, V., Seguin, P., 2015. Performance of low-cost open-top chambers to study long-term effects of carbon dioxide and climate under field conditions. *Agron. J. 107*, 916-920.

Mithöfer, A., Boland, W., 2012. Plant defense against herbivores: chemical aspects. *Annu. Rev. Plant. Biol. 63*, 431-450.

Miyagawa, H., 2009. Studies on nitrogen-containing secondary metabolites playing a defensive role in plants. J. Pestic. Sci. 34, 110-112.

Mochizuki, T., Amagai, T., Tani, A., 2018. Effects of soil water content and elevated CO_2 concentration on the monoterpene emission rate of *Cryptomeria japonica*. *Sci. Tot. Environ. 634*, 900-908.

Morison, J.I.L., Lawlor, D.W., 1999. Interactions between increasing CO_2 concentration and temperature on plant growth. *Plant. Cell. Environ. 22*, 659–682.

Myhre, G., Alterskjær, K., Stjern, C.W., Hodnebrog, Ø., Marelle, L., Samset, B.H., Sillmann, J., Schaller, N., Fischer, E., Schulz, M., Stohl, A., 2019. Frequency of extreme precipitation increases extensively with event rareness under global warming. *Sci. Rep. 9*, 16063.

Nakonechnaya, O.V., Gorpenchenko, T.Y., Voronkova, N.M., Kholina, A.B., Zhuravlev, Y.N., 2013. Embryo structure, seed traits, and productivity of relict vine *Aristolochia contorta* (Aristolochiaceae). *Flora. 208*, 293-297.

Nakonechnaya, O.V., Kholina, A.B., Koren, O.G., Zhuravlev, Y.N., 2012. Genetic diversity of a rare species *Aristolochia contorta* Bunge (Aristolochiaceae) in Primorsky Krai. *Russ. J. Genet. 48*, 152-162.

Nam, B.E., Park, H.J., Son, G.Y., Ki, J.G., 2020. An analysis of the genetic diversity of a riparian marginal species, *Aristolochia contorta*. *J. Wet. Res. 22*, 100-105.

National Institute of Biological Resources, 2012. *Red Data Book of Endangered Insects in Korea I*. Ministry of Environment, Seoul, Korea.

Nie, G., Hendrix, D.L., Webber, A.N., Kimball, B.A., Long, S.P., 1995. Increased Accumulation of carbohydrates and decreased photosynthetic gene transcript levels in wheat grown at an elevated CO_2 concentration in the field. *Plant. Physiol. 108*, 975-983.

Nishida, R., 2002. Sequestration of defensive substances from plants by Lepidoptera. *Annu. Rev. Entomol. 47*, 57-92.

Nishida, R., Fukami, H. 1989. Ecological adaptation of an Aristolochiaceae-feeding swallowtail butterfly, *Atrophaneura alcinous*, to aristolochic acids, *J. Chem. Ecol. 15*, 2549-2563.

Norris, T., Wilkinson, D., Lockwood, A., Belay, A., Colls, J.J., Bailey, B.J., 1996. Performance of a controlled-ventilation open-top chamber for climate change research. *Agr. Forest. Meteorol. 78*, 239-257.

Obata, T., 2019. Metabolons in plant primary and secondary metabolism. *Phytochem. Rev. 18*, 1483-1507.

Optiz, S.E.W., Müller, C., 2009. Plant chemistry and insect sequestration, *Chemoecology. 19*, 117.

Palacio, S., Maestro, M., Montserrat-Martí, G., 2007. Seasonal dynamics of non-structural carbohydrates in two species of Mediterranean sub-shrubs with different leaf phenology. *Environ. Exp. Bot. 59*, 34-42.

Paré, P.W., Tumlinson, J.H., 1999. Plant volatiles as a defense against insect herbivores. *Plant. Physiol. 121*, 325-331.

Park, H.J., Park, H., Son, G.Y., Nam, B.E., Kim, J.G., 2020. Biotic and abiotic effects on the growth and reproduction of *Aristolochia contorta*. *J. Wet. Res. 22*, 113-120.

Park, S.H., Nam, B.E., Kim, J.G., 2019. Shade and physical support are necessary for conserving the *Aristolochia contorta* population. *Ecol. Eng. 135*, 108-115.

Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst. 37*, 637-669.

Pennisi, E., 2020. Carbon dioxide increase may promote 'insect apocalypse'. *Science. 368*, 459.

Pérez-Bueno, M.L., Pineda, M., Díaz-Casado, E., Barón, M., 2015. Spatial and

temporal dynamics of primary and secondary metabolism in *Phaseolus vulgaris* challenged by *Pseudomonas syringae*. *Physiol. Plantarum. 153*, 161-174.

Pfahl, S., O'Gorman, P.A., Fischer, E.M., 2017. Understanding the regional pattern of projected future changes in extreme precipitation. *Nat. Clim. Change. 7*, 423-427.

Piessens, K., Adriaens, D., Jacquemyn, H., Honnay, O., 2009. Synergistic effects of an extreme weather event and habitat fragmentation on a specialised insect herbivore. *Oecologia. 159*, 117-126.

Pires, M.M., O'Donnell, J.L., Burkle, L.A., Díaz-Castelazo, C., Hembry, D.H., Yeakel, J.D., Newman, E.A., Medeiros, L.P., de Aguiar, M.A.M., Guimarães, Jr.P.R., 2020. The indirect paths to cascading effects of extinctions in mutualistic networks. *Ecology. 101*, e03080.

Piva, A., Bertrand, A., Bélanger, G., Castonguay, Y., Seguin, P., 2013. Growth and physiological response of timothy to elevated carbon dioxide and temperature under contrasted nitrogen fertilization. *Crop. Sci. 53*, 704-715.

Prinsloo, G., Nogemane, N., 2018. The effects of season and water availability on chemical composition, secondary metabolites and biological activity in plants. *Phytochem. Rev. 17*, 889-902.

Pritchard, S.H.G., Rogers, H.O.H., Prior, S.A., Peterson, C.T.M., 2001. Elevated CO_2 and plant structure: a review. *Glob. Change. Biol. 5*, 807-837.

Pugliese, A., 1988. Optimal resource allocation in perennial plants: a continuous-time model. *Theor. Popul. Biol. 34*, 215-247.

R Core Team, 2019. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ren, P., Néron, V., Rossi, S., Liang, E., Bouchard, M., Deslauriers, A., 2020. Warming counteracts defoliation-induced mismatch by increasing herbivore-

plant phenological synchrony. *Glob. Change. Biol. 26*, 2072-2080.

Rosenthal, G.A., Berenbaum, M.R., 2012. *Herbivores: Their Interactions with Secondary Plant Metabolites: Ecological and Evolutionary Processes.* Academic Press, USA.

Rosenzweig, C., Hillel, D., 1998. Climate change and the global harvest. In: *Potential Impacts of the Greenhouse Effect on Agriculture*. Oxford University Press, New York, USA.

Royer, M., Larbat, R., le Bot, J., Adamowicz, S., Robin, C., 2013. Is the C:N ratio a reliable indicator of C allocation to primary and defense-related metabolisms in tomato? *Phytochemistry. 88*, 25-33.

Sabelis, M.W., Takabayashi, J., Janssen, A., Kant, M.R., van Wijk, M., Sznajder, B., Aratchige, N.S., Lesna, I., Belliure, B., Schuurink, R.C., 2007. Ecology meets plant physiology: herbivore-induced plant responses and their indirect effects on arthropod communities. In: *Ecological Communities: Plant Mediation in Indirect Interaction Webs*. Cambridge University Press, New York, USA. pp. 188-217.

Sage, R.F., Sharkey, T.D., Seemann, J.R., 1989. Acclimation of photosynthesis to elevated CO_2 in five C_3 species. *Plant. Physiol. 89*, 590-596.

Samarakoon, A.B., Gifford, R.M., 1995. Soil water content under plants at high CO_2 concentration and interactions with the direct CO_2 effects: a species comparison. *J. Biogeogr. 22*, 193-202.

Sánchez-Bayo, F., Wyckhuys, K.A.G., 2019. Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv. 232*, 8-27.

Sax, D.F., Early, R., Bellemare, J., 2013. Niche syndromes, species extinction risks, and management under climate change. *Trends. Ecol. Evol. 28*, 517-523.

Scala, A., Allmann, S., Mirabella, R., Haring, M.A., Schuurink, R.C., 2013. Green leaf volatiles: a plant's multifunctional weapon against herbivores and pathogens.

Int. J. Mol. Sci. 14, 17781-17811.

Schädler, M., Roeder, M., Brandl, R., Matthies, D., 2007. Interacting effects of elevated CO_2, nutrient availability and plant species on a generalist invertebrate herbivore. *Glob. Change. Biol. 13*, 1005-1015.

Schäfer, M., Brütting, C., Baldwin, I.T., Kallenbach, M., 2016. High-throughput quantification of more than 100 primary- and secondary-metabolites, and phytohormones by a single solid-phase extraction based sample preparation with analysis by UHPLC–HESI–MS/MS. *Plant. Methods. 12*, 30.

Schwachtje, J., Baldwin, I.T., 2008. Why does herbivore attack reconfigure primary metabolism? *Plant. Physiol. 146*, 845-851.

Shin, Y.H., 1974. Life history of *Sericinus telamon* Donovan in Korea. Master Thesis, Kyung Hee University.

Singer, M.C., Parmesan, C., 2010. Phenological asynchrony between herbivorous insects and their hosts: signal of climate change or pre-existing adaptive strategy? *Philos. T. Roy. Soc. B. 365*, 3161-3176.

Singh, S.K., 2018. Explorations of plant's chemodiversity: role of nitrogen-containing secondary metabolites in plant defense. In: Singh, A., Singh, I., (eds) *Molecular Aspects of Plant-Pathogen Interaction*. Springer, Singapore. pp. 309-332.

Smith, T.M., Shugart, H.H., Bonan, G.B., Smith, J.B., 1992. Modeling the potential response of vegetation to global climate change. Adv. Ecol. Res. 22, 93-98.

Solórzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr. 14*, 799-801.

de Souza A.P., Gaspar, M., da Silva, E.A., Ulian, E.C., Waclawovsky, A.J., Nishiyama, Jr.M.Y., dos Santos, R. V., Teixeira, M.M., Souza, G.M., Buckeridge, M.S., 2008. Elevated CO_2 increases photosynthesis, biomass and productivity, and modifies gene expression in sugarcane. *Plant. Cell.*

Environ. 31, 1116-1127.

Steinbrenner, A.D., Gómez, S., Osorio, S., Fernie, A.R., Orians, C.M., 2011. Herbivore-induced changes in tomato (*Solanum lycopersicum*) primary metabolism: a whole plant perspective. *J. Chem. Ecol. 37*, 1294-1303.

Suh, H.W., Hyun, S.-H., Kim, S.-H., Lee, S.-Y., Choi, H.-K., 2013. Metabolic profiling and enhanced production of phytosterols by elicitation with methyl jasmonate and silver nitrate in whole plant cultures of *Lemna paucicostata*. *Process. Biochem. 48*, 1581-1586.

Sviridov, A.V., 1983. *Sericinus montela* Gray 1853. In: *Red Book of Russian Soviet Federative Socialist Republic: Animals*. Rosagropromizdat, Moscow, Russia.

Tahir, K.E.H.E., 1991. Pharmacological actions of magnoflorine and aristolochic acid-1 isolated from the seeds of *Aristolochia bracteate*. *Int. J. Pharmacogn. 29*, 101-110.

Taub, D.R., Miller, B., Allen, H., 2008. Effects of elevated CO_2 on the protein concentration of food crops: a meta-analysis. *Glob. Change. Biol. 14*, 565-575.

Theis, N., Lerdau, M., 2003. The evolution of function in plant secondary metabolites. *Int. J. Plant. Sci. 164*, S93-S102.

Thomson, A.M., Calvin, K.V., Smith, S.J., Kyle, G.P., Volke, A., Patel, P., Delgado-Arias, S., Bond-Lamberty, B., Wise, M.A., Clarke, L.E., James, A.E., 2011. RCP4.5: a pathway for stabilization of radiative forcing by 2100. *Clim. Change. 109*, 77.

Trenberth, K.E., 2011. Changes in precipitation with climate change. *Clim. Res. 47*, 123-138.

Trowbridge, A.M., 2014. Evolutionary ecology of chemically mediated plant-insect interactions. In: *Ecology and the Environment*, pp. 143-176.

Tsai, Y.C., Delk, N.A., Chowdhury, N.I., Braam, J., 2007. *Arabidopsis* potential

calcium sensors regulate nitric oxide levels and the transition to flowering. *Plant. Signal. Behav. 2*, 446-454.

van Velzen, E., Etienne, R.S., 2013. The evolution and coexistence of generalist and specialist herbivores under between-plant competition. *Theor. Ecol. 6*, 87-98.

Veteli, T.O., Kuokkanen, K., Julkunen-Tiitto, R., Roininen, H., Tahvanainen, J., 2002. Effects of elevated CO_2 and temperature on plant growth and herbivore defensive chemistry. *Glob. Change. Biol. 8*, 1240-1252.

Voronkova, N.M., Kholina, A.B., Koldaeva, M.N., Nakonechnaya, O., Nechaev, V.A., 2018. Morphophysiological dormancy, germination, and cryopreservation in *Aristolochia contorta* seeds. *Plant. Ecol. Evol. 151*, 77-86.

Wagner, D.L., 2020. Insect declines in the Anthropocene. *Annu. Rev. Entomol. 65*, 457-480.

Walter, J., Hein, R., Auge, H., Beierkuhnlein, C., Löffler, S., Reifenrath, K., Schädler, M., Weber, M., Jentsch, A., 2012. How do extreme drought and plant community composition affect host plant metabolites and herbivore performance? *Arthropod-Plant. Inte. 6*, 15-25.

Walther, G.R., 2010. Community and ecosystem responses to recent climate change. *Philos. T. Roy. Soc. B. 365*, 2019-2024.

Wang, Y.T., 2007. Potassium nutrition affects Phalaenopsis growth and flowering. *Hortscience. 42*, 1563-1567.

War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S., Sharma, H.C., 2012. Mechanisms of plant defense against insect herbivores. *Plant. Signal. Behav. 7*, 1306-1320.

Weaver, D.K., McFarlane, J.E., 1990. The effect of larval density on growth and development of *Tenebrio molitor*. *J. Insect Physiol. 36*, 531-536.

Weiner, J., 1988. The influence of competition on plant reproduction, *Plant*

Reproductive Ecology: Patterns and Strategies, Oxford University Press, NY, USA, pp. 228-245.

Weis, A.E., Abrahamson, W.G., 1985. Potential selective pressures by parasitoids on a plant-herbivore interaction. *Ecology. 66*, 1261-1269.

Welti, E.A.R., Roeder, K.A., de Beurs, K.M., Joern, A., Kaspari, M., 2020. Nutrient dilution and climate cycles underlie declines in a dominant insect herbivore. *PNAS, 117*, 7271-7275.

Wen, Y., Su, T., Tang, J., Zhang, C., Wang, X., Cai, S., Li, X., 2006. Cytotoxicity of phenanthrenes extracted from *Aristolochia contorta* in human proximal tubular epithelial cell line. *Nephron. Exp. Nephrol. 103*, e95-e102.

Wilson, R.J., Maclean, I.M.D., 2011. Recent evidence for the climate change threat to Lepidoptera and other insects. *J. Insect. Conserv. 15*, 259-268.

Wink, M., 1999. *Functions of plant secondary metabolites and their exploitation in biotechnology*. Sheffield Academic Press, UK.

Wink, M., 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry. 64*, 3-19.

Witzgall, P., Ansebo, L., Yang, Z., Angeli, G., Sauphanor, B., Bengtsson, M., 2005. Plant volatiles affect oviposition by codling moths. *Chemoecology. 15*, 77-83.

Wu, J., Baldwin, I.T., 2010. New insights into plant responses to the attack from insect herbivores. *Annu. Rev. Genet. 44*, 1-24.

Wullschleger, S.D., Norby, R.J., Hendrix, D.L., 1992. Carbon exchange rates, chlorophyll content, and carbohydrate status of two forest tree species exposed to carbon dioxide enrichment. *Tree. Physiol. 10*, 21-31.

Xu, H., Xie, H., Wu, S., Wang, Z., He, K., 2019. Effects of elevated CO_2 and increased N fertilization on plant secondary metabolites and chewing insect fitness. *Front. Plant. Sci. 10*, 739.

Yang, L., Wen, K., Ruan, X., Zhao, Y., Wei, F., Wang, Q., 2018. Response of plant secondary metabolites to environmental factors. *Molecules. 23*, 762.

Yang, L.H., Cenzer, M.L., Morgan, L.J., Hall, G.W., 2020. Species-specific, age-varying plant traits affect herbivore growth and survival. *Ecology. 101*, e03029.

Yang, L.H., Rudolf, V.H.W., 2010. Phenology, ontogeny and the effects of climate change on the timing of species interactions. *Ecol. Lett. 13*, 1-10.

Yang, T., Poovaiah, B.W., 2003. Calcium/calmodulin-mediated signal network in plants, *Trends. Plant. Sci. 8*, 505-512.

Yin, J., Sun, Y., Wu, G., Parajulee, M.N., Ge, F., 2009. No effects of elevated CO2 on the population relationship between cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), and its parasitoid, *Microplitis mediator* Haliday (Hymenoptera: Braconidae). *Agr. Ecosyst. Environ. 132*, 267-275.

Yuan, Y., Liu, Y., Wu, C., Chen, S., Wang, Z., Yang, Z., Qin, S., Huang, L., 2012. Water deficit affected flavonoid accumulation by regulation hormone metabolism in *Scutellaria baicalensis* Georgi roots*. Plos One. 7*, e42946.

Zangerl, A.R., 2003. Evolution of induced plant responses to herbivores. *Basic. Appl. Ecol. 4*, 91-103.

Zavala, J.A., Nabity, P.D., DeLucia, E.H., 2013. An emerging understanding of mechanisms governing insect herbivory under elevated CO_2. *Annu. Rev. Entomol. 58*, 79-97.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Van 't Riet, K., 1990. Modeling of the bacterial growth curve. *Appl. Environ. Microb. 56*, 1875-1881.

국문초록

기후변화가 진행됨에 따라 나타나는 빠른 환경 변화로 인해 곤충을 포함한 생물다양성의 위기가 도래할 가능성이 높다. 생태계 내에서 곤충의 생태학적 · 생물학적인 가치를 생각해 볼 때, 기후변화가 곤충에 미칠 수 있는 영향을 구체적으로 규명하기 위한 연구가 필요하다. 이러한 관점에서 식물은 곤충에 주요한 영향을 미칠 수 있는 요인이므로 기후변화에 대한 식물의 반응을 이해하기 위한 노력이 선행되어야 한다. 지금까지 이를 해결하기 위한 연구가 다수 진행되었으며, 이를 토대로 기후변화로 인한 식물의 반응과 그로 인한 곤충 군집의 변화에 대한 가설이 제안되었다. 그러나, 기후변화는 복잡한 환경 요인의 변화를 동반하며 그에 대한 식물의 반응 또한 종의 특성에 따라 다른 양상으로 나타날 수 있어, 기후변화에 대한 현재의 주요 예측은 여전히 더 많은 실험적 증거와 증명을 필요로 한다. 특히, 기후변화가 식물과 곤충의 상호작용에 미치는 영향에 주요한 변화를 일으킬 것으로 예상되는 강수 빈도의 증가와 식물과 곤충의 계절성에 대한 연구는 여전히 미진한 실정이다. 따라서, 본 연구에서는 우리나라 고유종인 쥐방울덩굴(*Aristolochia contorta*)과 이를 유일한 기주식물로 활용하는 특이적 초식 곤충인 꼬리명주나비(*Sericinus montela*)를 활용하여 환경 요인이 식물-곤충 상호작용에 미치는 영향을 파악하고자 하였다. 환경 요인이 식물의 생육과 방어 작용에 미치는 영향과 그로 인해 나타나는 초식 곤충의 생육 변화의 관계를 규명하기 위하여 본 연구에서는 세 가지의 주요한 실험을 실행하였다. 우선, 다양한 환경 요인의 기주식물에 대한 영향의 가능성을 타진하기 위하여 쥐방울덩굴의 서식지에 대한 현장 조사를 수행하였다. 또한, 이산화탄소 농도의 상승과 강수 빈도의 증가가 식물의 생육과 방어 작용에 미치는 영향 및 초식 곤충에 대한 연쇄적인 효과를 파악하고, 기후변화에 따라 나타나는 식물-

곤충 상호작용 변화의 계절적인 동태를 파악하기 위하여 상부개방형온실(open-top chamber)을 활용한 두 개의 메조코즘 실험을 진행하였다. 줄기 길이와 잎의 수를 측정하여 식물의 생장 양상을 관찰하였으며, 초식 곤충의 상대생장률(relative growth rate)을 기반으로 기후변화에 따른 식물의 반응이 곤충에 미치는 영향을 파악하였다. 추가적으로, 식물의 잎의 영양 가치를 평가하기 위하여 탄소: 질소 비율(C: N ratio)과 1차 대사산물을 분석하였고, 식물 잎에서 나타나는 화학적 반응을 비교하기 위하여 2차 대사산물을 분석하였다. 현장 조사 결과, 쥐방울덩굴의 생육은 다양한 생물적 요인과 비생물적 요인에 영향을 받을 수 있는 것으로 드러났다. 특히, 초식 곤충으로 인한 섭식 스트레스와 종간 경쟁으로 인한 스트레스, 토양 내 양이온 함량이 주요한 요인으로 확인되었다. 기후변화에 따른 식물의 반응의 관점에서는, 이산화탄소 농도의 상승이 식물의 광합성을 억제하여 생육을 감소시키고 식물의 방어 작용은 증진시켰다. 이러한 증진된 식물의 방어 작용에 따라 초식 곤충의 생육은 억제되었다. 이와 달리, 강수 빈도의 증가는 이러한 높은 농도의 이산화탄소의 영향을 부분적으로 완화하여, 초식 곤충의 생장을 증가시켰다. 더불어, 이러한 이산화탄소 농도의 상승과 강수 빈도의 증가가 잎의 영양 가치와 식물의 상시 방어(constitutive defense)에 미치는 영향은 시기에 따라 다르게 나타났다. 증가된 강수 빈도는 영양 가치를 증진시켰으며, 이러한 영향은 식물의 생육 기간의 중간에 가장 크게 나타났다. 반면, 상승된 이산화탄소 농도는 영양 가치를 감소시키고 상시 방어를 증진시켰으며, 식물의 생육 기간의 종료 시기에 가장 크게 확인되었다. 이러한 시기적으로 일치하지 않는 변화에 따라 특이적 초식 곤충과 비특이적 초식 곤충의 생장 양상 또한 계절적 변동을 나타낸 것으로 보인다. 결론적으로, 강수 빈도의 증가보다 이산화탄소 상승이 미치는 더 주요한 영향을 미치는 것을 고려해볼 때, 기주식물의 질과 양이 모두 감소할 수 있으며, 이에 따라 그것을 이용하는 특이적 초

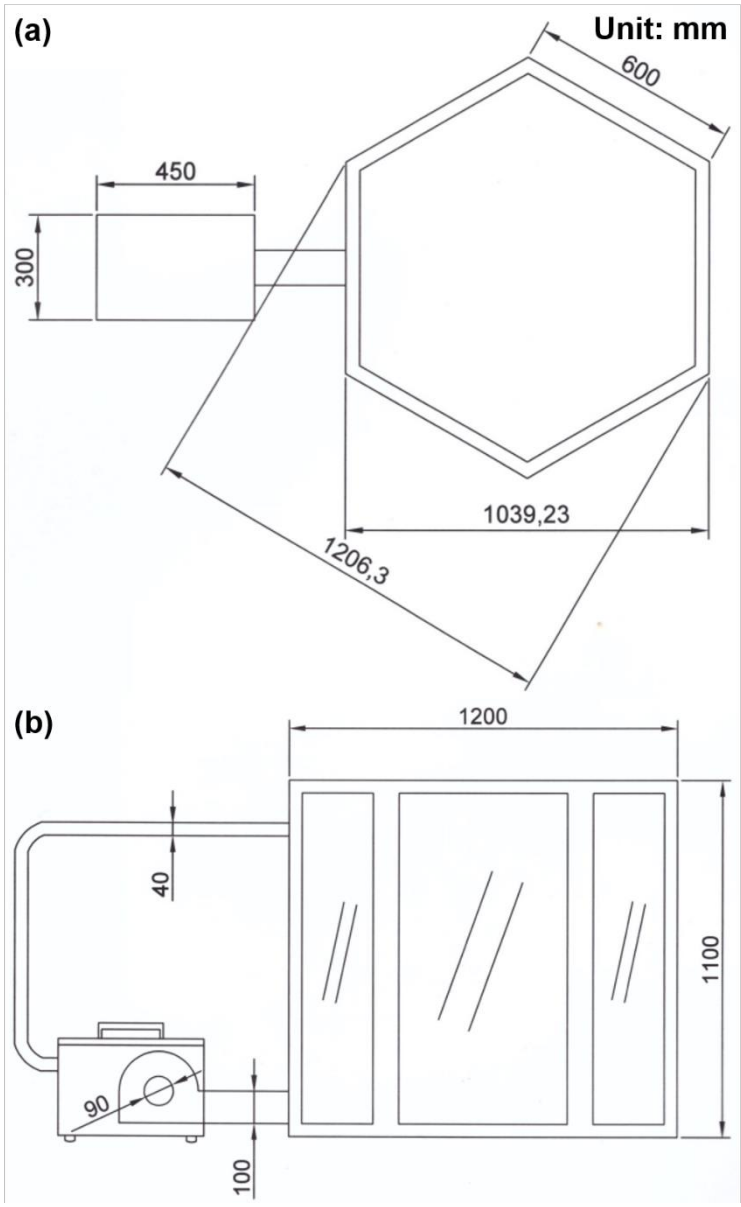
식 곤충의 생장도 억제될 수 있을 것으로 생각된다. 이러한 결과는 기후변화가 곤충 군집에 부정적인 영향을 미칠 것이라는 기존에 제시된 가설과 같은 결과를 예상하지만 그 과정은 다르게 나타날 수 있음을 시사한다. 그러나, 기후변화로 인한 환경 변화가 시기적으로 다른 영향을 미친다는 것을 고려해볼 때, 강수빈도의 증가가 초식 곤충의 생활사 중 특정 시기의 생장을 증가시킴으로써 이산화탄소 증가의 부정적인 효과를 일부 상쇄할 수 있을 것으로 판단된다. 뿐만 아니라, 본 연구의 결과는 식물-곤충 상호작용에서 식물의 종특이적인 반응과 기존에 고려되지 않았으나 주요한 영향력을 가지고 있을 수 있는 환경 요인을 고려함으로써, 기후변화가 식물과 초식 곤충의 상호작용에 미치는 영향을 종합적으로 이해하는 데에 기여할 것으로 예상된다. 또한, 식물과 곤충의 생활사적 특성을 고려하는 것을 통해 기후변화 환경에서 식물-곤충 상호작용 변화의 더욱 정확한 예측을 가능하게 할 것으로 기대된다.

주요어: 계절성, 기후변화, 식물-곤충 상호작용, 식물 구성 방어, 영양 가치, 특이적 초식 곤충

학번: 2017-23946

Appendices

Appendix 1. The blueprint of an open-top chamber



Appendix Figure 1-1. The blueprint of open-top chamber. This blueprint and actual device were made by Kukje Engineering (Korea). (a) Top view, (b) Side view

Appendix Table 1-1. Materials for an open-top chamber and an air circulation box.

Product Name		Specification
Chamber	Acrylic plate	Clean
	Stainless plate	SUS #304 (Stainless)
Air Circulation Box	Blower motor	1/3 Hp, over 566 $m \cdot sec^{-1}$
	Pre-filter	Nylon-wooven fabric (100 mm × 150 mm)
	Jabara hose	40 mm × 3 m
	Air inflow pipe	100 mm × 6 m (T=1, 45%)
	Caster	Urethane rubber 2”
	Power supply	AC 220V, 60Hz, 1ϕ

<Detail materials and specifications of CO_2 controlling system>

- CO_2 sensor-transmitter (SH-VT260, Soha-tech, Korea) coupled with a CO_2 controller (0 - 2,000 ppm_{CO2}, SH-MVG260, Soha-tech, Korea) was used for controlling CO_2. This device additionally needs a solenoid valve, an individual CO_2 gas tank with a gas regulator, and some urethane hoses with following specifications.

(1) Solenoid valve: 220 V, 8 A (1/4”), N/C, one-touch fitted with 6 mm PC

(2) CO_2 gas tank (99.999%, 40 L, Daeseong Gas, Korea) with a gas regulator (Victor, USA, purchased via Daeseong Gas, Korea) one-touch fitted with 6 mm PC.

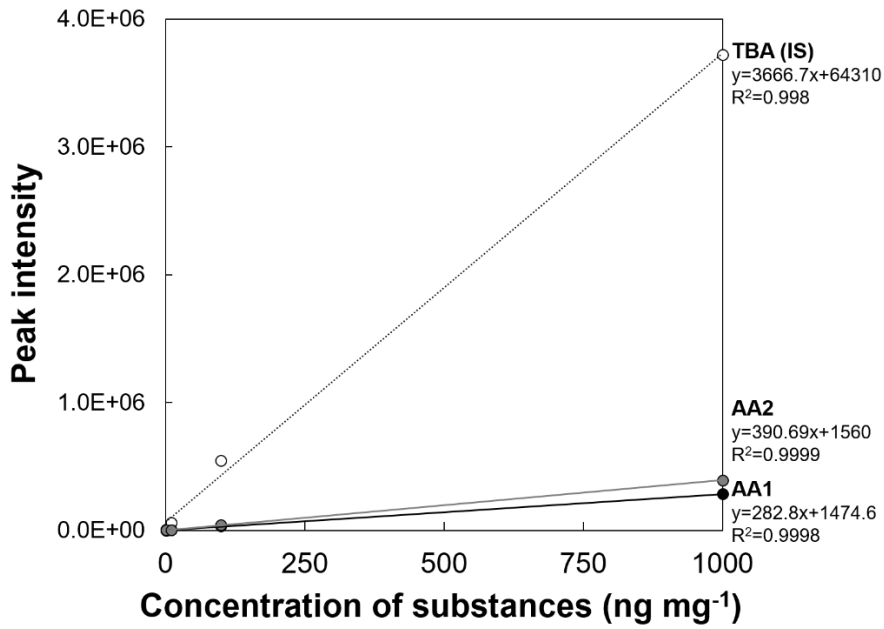
(3) 6 mm urethane rubber hose (for both input and output of solenoid valve)

* A 1 mL micropipette tip (Eppendorf, Germany) filled with cotton was attached at the end of outflow hose to reduce the output gas volume.

* The end of outflow hose should be located at the front of the air inlet of fan in an air circulation box.

Appendix 2. Response curve for the exact quantification of aristolochic acid 1 (AA1) and 2 (AA2)

Although the concentrations of different compounds are the same, their peak intensity measured by the instrument could be distinct according to chemical characteristics of each compound. Therefore, it is necessary to get a reaction curve with the peak intensity values of several known concentrations for standards of each compound. In order to get exact amounts of AA1 and AA2, which are the typical secondary metabolites of *A. contorta*, we measured the peak intensity of 1, 10, 100, and 1,000 ng·mg^{-1} of TBA (tribenzylamine, an internal standard used in this research), AA1, and AA2. Then, we calculated the exact amounts of AA1 and AA2 with the slope of each standard curve after the normalization of peak intensity (Appendix Figure 2-1).



Appendix Figure 2-1. The response curve for TBA, AA1, and AA2.

Appendix 3. Primary metabolites analysis method

1) Leaf samples frozen at -80°C were ground and dried in a frozen state before extraction.
2) 10 mg of freeze-dried samples were aliquoted each in 1.5 mL tube (Eppendorf, Germany).
3) 1 mL of methanol (HPLC grade, Sigma-Aldrich, USA) was added to extract metabolites.
4) Vortexed for 40 sec and sonicated for 40 min (50-60% of magnitude)
5) Supernatants were collected and filtered through 0.45 μm PTFE syringe filters after sonication.
6) 100 mL of each filtered sample was transferred into amber GC vials (Agilent, USA).
7) Samples were dried with nitrogen (N_2) gas for 5 min in an evaporator
8) Oximation process was performed with following solutions: 30 μl of 20,000 $\mu g \cdot mL^{-1}$ methoxylamine hydrochloride in pyridine (Sigma-Aldrich, USA), 50 μl of N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (Sigma-Aldrich, USA), and 10 μl of 300 $\mu g \cdot mL^{-1}$ of 2-chloronaphthalene (Sigma-Aldrich, USA) in pyridine as an internal standard (IS).
9) Samples were incubated in a 65°C heat block for 1 hour.
10) Extractions were analyzed using a Shimadzu gas chromatography system (GC-MS QP2020, Shimadzu Corp., Japan).
11) GC-MS condition of metabolites was referred to Suh et al. (2013) with minor modifications as follows.

<Detail condition of GC-MS>

- The temperature of GC inlet: 250°C, wet
- Injection volume: 1.0 μL
- Split ratio: 1:5, using helium as the carrier gas at a constant-flow rate of 1.0 $mL \cdot min^{-1}$.
- Set range of mass: 50~600 Da
- Column: A Rtx-5MS column (Shimadzu, Corp., Japan) with 30 m × 0.25 mm I.D. × 0.25 μm d_f dimensions.
- Oven program (1) first set to 70°C, held for 5 min
  (2) ramped up to 130°C at 15°C·min^{-1}.
  (3) 160°C at 4°C·min^{-1} was held for 15 min
  (4) 300°C at 10°C·min^{-1} was held for 9 min
- A total of running time: 54.5 min
- National Institute of Standards and Technology (NIST) mass spectral search library was used for identification of metabolites in samples.
- A match quality greater than 80% was selected in the peak assignment
- Normalization was performed by dividing the peak area of each compound by that of the IS to compare relative abundance of selected metabolites in each sample.

Appendix 4. Phytohormone analysis method

1) Leaf samples frozen at -80°C were ground in a frozen state before extraction.
2) 50 mg of ground samples were aliquoted each in 2 mL tube (Eppendorf, Germany).
3) Two steel balls of 3 mm diameter and 1 mL of ethylacetate (HPLC grade, Sigma-Aldrich, USA) spiked with labeled phytohormones as IS: D_6-ABA, D_4-SA, and JA-$^{13}C_6$-Ile in 20 ng·uL^{-1} were added for each sample.
4) Vortexed using Tissuelyser II (Qiagen, Germany) with 26 stroke·sec^{-1} for 2 min.
5) Centrifuged at 13,000 rpm for 20 min at 4°C, and supernatants were transferred to new 2 mL tube each (Eppendorf, Germany).
6) The extraction process was repeated by adding 0.5 mL of ethylacetate without IS
7) Supernatants were combined after centrifuge at 13,000 rpm for 20 min at 4°C
8) Evaporated using HyperVAC-MAX (Hanil Scientific Inc., Korea) until dryness at 30°C.
9) After confident dryness, 500 uL of 70% methanol (HPLC grade, Sigma-Aldrich, USA) were then added to dissolve metabolites, and vortexed with Tissulyser II with 26 stroke·sec^{-1} for 1 min.
10) Centrifuged at 13,000 rpm for 10 min at 4°C, and 400 uL of dissolved samples were transferred into a HPLC vial each (Agilent, 2 mL, USA).
11) The extracts were analyzed by ultra-high-performance liquid chromatography (UHPLC)-triple quadrupole mass spectrometer

(TQMS), referring to Schäfer et al. (2016) with little modifications as follows.

<Detail condition of UHPLC-TOFMS>

- Instrument: LCMS-8050, Shimadzu, Japan
- Injection volume: 1 μL
- Column: C18 column (UPLC BEH, 1.7 μm particle size, 100 mm length X 2.1 μm inner diameter, Waters, Ireland)
- Solvent A: deionized water containing 0.1% (v/v) acetonitrile and 0.5% formic acid / Solvent B: 100% methanol
- Gradient elution program:

  0-0.1 min, 5% B; 0.1-7.0 min, linear gradient 95% B; 7.01-12 min, 100% B; and re-equilibration at 5% B for 3 min
- Flow rate: 400 $mL \cdot min^{-1}$
- Amounts of eluted analytes were determined by an ESI-TOF mass spectrometer (Bruker Daltonic, Bremen, Germany) with the molecular mass of ionized molecular fragments of standard phytohormones.

  (JA: M.W. = 210.27 $g \cdot mol^{-1}$, precursor ion m/z = (-) 215.30, product ion m/z = 59.05 and 40.90; SA: M.W. = 138.12 $g \cdot mol^{-1}$, precursor ion m/z = (-) 141.20, product ion m/z = 97.10 and 69.10; ABA: M.W. = 264.32 $g \cdot mol^{-1}$, precursor ion m/z = (-) 269.30, product ion m/z = 159.25 and 225.25)
- Capillary voltage: 3.0 kV
- Dry gas flow rate: 10.0 $L \cdot min^{-1}$
- This analysis was done by the KAIST Bio-core Center.

Appendix 5. Secondary metabolites analysis method

1) Leaf samples frozen at -80°C were ground in a frozen state before extraction.
2) 50 mg of ground samples were aliquoted each in 2 mL tube (Eppendorf, Germany).
3) Two steel balls of 3 mm diameter and 1 mL of 40% methanol (HPLC grade, Sigma-Aldrich, USA) containing 0.1 $\mu g \cdot mL^{-1}$ of tribenzylamine (TBA) (≥ 99.0%, Sigma, USA) as an internal standard (IS) were added to each sample.
4) Vortexed using Tissuelyser II (Qiagen, Germany) with 26 $stroke \cdot sec^{-1}$ for 2 min.
5) Centrifuged at 13,000 rpm for 20 min at 4°C.
6) Transformed about 850 µL of the supernatant to a 1.5 mL tube (Eppendorf, Germany).
7) Centrifuged at 13,000 rpm for 20 min at 4°C.
8) Collected supernatants and filtered through 0.22 µm PTFE syringe filters.
9) (Optional) Overnighted the samples at -20°C and then centrifuged again at the same condition.
10) 200 µL of each supernatant was transferred into LC vials with inserts of 250 µL (Agilent, 2 mL, USA).
11) The secondary metabolites were measured in the extraction using an ultra-performance liquid chromatography (UPLC)-quadrupole orthogonal time of flight mass spectrometer (qTOFMS) (Waters ACQUITY UPLC, Micromass Q-Tof micro, Waters, USA), following the method of Mao et al. (2017) with minor modifications as follows.

<Detail condition of UHPLC-TOFMS>

- Injection volume: 2 μL
- Column: UHPLC C18 column (2.1 mm × 100 mm, I.D., 1.7 μm, ACQUITY UHPLC® HSS, Waters, USA), coupling with a C18 pre-column (2.1 mm × 5 mm, I.D., 1.7 μm, Vanguard TM HSS, Waters), maintained at 40°C.
- Solvent A: distilled water containing 0.2% (v/v) formic acid

  Solvent B: acetonitrile with 0.2% formic acid
- Flow rate: 0.4 $mL \cdot min^{-1}$
- Gradient elution program:

  0 min, 10% B; 2 min, 50% B; 7 min, 95% B; 11 min, 95% B, 15 min, 45%
- Eluted compounds were detected from m/z 100 to 1000 using Xevo G2-XS QTOF mass spectrometer (Waters, Manchester, UK) which was connected to an electrospray ionization (ESI) source interface with positive mode using the following instrument setting:

  (1) Drying gas (N_2) flow rate: 8 $mL \cdot min^{-1}$

  (2) Drying gas temperature: 300°C

  (3) Nebulizer pressure: 45 psi

  (4) Capillary voltage: 3,500 V

  (5) Nozzle voltage: 500 V / Fragmentor: 120 V

  (6) Fixed collision energies: 15 and 30 eV, respectively
- Data acquisition was achieved using Masslynx v 4.1 (Waters, Milford, MA, USA).