Phyton (Austria) Special issue: "APGC 2004"	**Vol. 45**	Fasc. 4	(163)-(168)	1.10.2005

Characteristics of Defense Chemicals of Mountain Alder Seedlings Raised under Elevated CO_2 and Nitrogen Supply

By

T. KOIKE [1], T. SHIBATA [2], T.T. LEI [1][3], S. MATSUKI [2][4], H. TOBITA [5], M. KITAO [5]
N. YAMASHITA [5], A.M.QUORESHI [5] & Y. MARUYAMA [5]

Key words: High CO_2, nitrogen deposition, alder (*Alnus hirsuta*), defense chemicals, soil fertility.

Summary

KOIKE T., SHIBATA, T., MATSUKI S., LEI T.T., TOBITA H., KITAO M. QUORESHI A.M. & MARUYAMA Y. 2005. Characteristics of defense chemicals of mountain alder seedlings raised under elevated CO_2 and nitrogen supply. - Phyton (Horn, Austria) 45 (4): (163)-(168).

Japanese mountain alder (*Alnus hirsuta*) seedlings were raised in a factorial combination of two levels of CO_2 and two levels of nutrient to examine their foliar defense traits. Alders are actinorrhizal plants and are capable of fixing atmospheric nitrogen through root nodules formed by the actinomycete *Frankia* sp. We examined the defense capacity of alder in relation to atmospheric CO_2 enrichment and soil fertility as simulated acid nitrogen deposition. In order to evaluate the degree of defense in alder leaves under these conditions, a bioassay study was carried out with wild silkworm, "Erisan" (*Samia cynthin ricini*). While the longevity of Erisan fed with leaves grown at high CO_2 and infertile condition was greater than in other treatments, all individuals tested, however, were dead before they reached the pupal stage. The results suggest firstly that the activity of the symbiotic *Frankia* sp. may have been enhanced by the greater amount of photosynthates that they received from the host plants (i.e. alder) at high CO_2. In this process, the *Frankia* sp. would increase the amount of nitrogen to leaves, which improved the food quality for Erisan. However, the reason that Erisan failed to become pupa could be that some essential elements in alder leaves might be insufficient or that the presence of defense chemicals as total phenolics in leaves might have interfered with Erisan's development.

---

[1] Hokkaido University Forests, Sapporo 060-0809, Japan. Fax: 81-11-706-3450, e-mail: tkoike@exfor.agr.hokudai.ac.jp

[2] Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan.

[3] Faculty of Science and Technology, Ryukoku University, Otsu 520-2194, Japan.

[4] JSPS fellow in Hokkaido Forestry Research Institute, Bibai 079-0198, Japan.

[5] Forestry and Forest Products Research Institute, Sapporo 062-8512, Japan.

Introduction

Mountain alder (*Alnus hirsuta*) is a common early successional tree species broadly distributed in northern Japan. This species is characterized by a high leaf nitrogen content because of its symbiosis with the actinomycete *Frankia* sp. (KOIKE & al. 1997). Therefore, alder leaves are usually grazed by several herbivores, especially leaf beetles (*Agelastica coerulea*) which can result in reducing the crown to a skeleton shape (e.g. BAUER & al. 1991).

Atmospheric CO_2 $[CO_2]^a$ is increasing yearly and the amount of atmospheric nitrogen deposition is also increasing. How will these changes affect the nature of production of defense chemicals in plants and consequently, the grazing damage caused by herbivores in the future? In general, the chemicals responsible for enhancing defense capacity of leaves increase in plants grown under infertile soil condition (BRYANT & al. 1983, COLEY & al. 1985). Therefore, it is expected that leaves of alder grown in infertile soil conditions would be more resistant against insect herbivores because of the increased production of defense chemicals. Such defense chemicals include condensed tannin and several phenolics. Also, the capacity of chemical defense of broadleaf trees is usually higher at elevated CO_2 $[CO_2]^e$ conditions (LINDROTH 1995, KOIKE & al. 2003). Therefore, it is predicted that the defense capacity of alder in infertile soil combined with $[CO_2]^e$ in the future will be high. However, low soil fertility could also change in the future because of the greater atmospheric nitrogen deposition, which usually acts as a fertilizer. In this study, we ask what would be the effect on defense capacity of alder seedlings from different combinations of CO_2 enrichment and soil fertility ?

In order to address the question, we examined the defense capacity of alder seedlings raised under $[CO_2]^e$ and $[CO_2]^a$ in combination with two nutrient regimes. Alder leaves taken from the treatment plants were fed to wild silkworm in a bioassay to evaluate the capacity of chemical defense in alders against herbivores.

Material and Methods

Plant material

Individual two-year-old seedlings of mountain alder (*Alnus hirsuta*) were planted in 5 liter pots filled with Kanuma pumices soil and clay soil (2:1 in volume). Four plants were used in each of the four factorial combination of two levels each of CO_2 and soil fertility. Alder is a N_2-fixing plant that forms a symbiotic association in its roots with an actinomycete of the genus *Frankia* sp. The nutrient condition of the seedlings was regulated by the application frequency of liquid fertilizer solution (Nitrogen 140 mg per application, N:P:K=4:3:2, Hyponex, U.S.) at once a week i.e. fertile (+N) or once a month i.e. infertile (-N). Six phytotron rooms (located at Forestry & Forest Products Research Institute in Sapporo, Japan) were used for CO_2 regulation where three chambers were allocated to each of 72 Pa and 36 Pa CO_2 concentrations. Moreover, the phytotrons were kept free of insects to avoid the occasion of induced defense in alder seedlings. Gas exchange measurements and bioassay began after the plants were grown their respective treatments for ca. 65 days.

Measurements

The gas exchange rate of 60 day-old alder leaves was measured with an open flow system (LI-6400, LiCor, Lincoln, NE, USA). Chlorophyll content of leaves was assessed by a SPAD (Type

502[?], Minolta, Tokyo, Japan). After the measurements, leaves were weighted for fresh weight and then oven dried at 60 °C for 48 hrs. The dried materials were used for the measurement of nitrogen content using a NC analyzer (NC-900, Shimadzu, Kyoto, Japan). The amount of total phenolics and condensed tannin were detected using the Folin-Ciocateus' method and sulfuric acid method (MATSUKI & al. 2004), respectively. LMA (leaf mass per area) was determined and used as an indicator of leaf toughness. Finally, the root system was examined for the number of nodules produced per plant and the fresh weight of the total root mass was taken.

A feeding experiment was carried out using "Erisan" i.e. a wild silkworm (*Samia cynthia ricini*), a generalist herbivore under a non-choice method for the evaluation of relative defense capacity in alder leaves. Erisans were kept at 25 °C, 75 % RH and 16 hr (light) and fed 30-40 days old leaves treated in each condition. Six wild silkworms were used in each feeding trial. Each feeding trial was replicated three times. We weighed each Erisan larva at two-day intervals and recorded its developmental stage (SHIBATA & al. 2004).

The results were evaluated by two-way analysis of variance (ANOVA) for each soil conditions. Systat (ver.11, SYSTAT Software, Inc.2004) was used for ANOVA.

Results and Discussion

The longevity ranking of Erisan (wild silkworm) larvae in the order from long to short was $[CO_2]^a$-N, $[CO_2]^a$+N, $[CO_2]^e$-N and $[CO_2]^e$+N (Fig.1). This result suggests that the amount of carbon-based chemicals for defense increased under $[CO_2]^e$. Survival (LD_{50}) of the larvae showed a similar tendency (data not shown). LMA as an indicator of physical defense in all treatments showed a similar value of 3.5 $mg \cdot cm^{-2}$. Therefore, chemical defense appears to have a more important role than physical defense (Fig. 2). There was no difference in total phenolics among treatments but the concentration of condensed tannin increased at $[CO_2]^e$ only +N. Even though alder leaves have less condensed tannin as compared with the other tree species, such as birch or maple (SHIBATA & al. 2004), all larvae tested were dead before they became pupae. To develop into pupae, a number of essential elements are required but the leaves may have contained insuffient quantities of these. Therefore, we should further examine the chemical composition of alder leaves. It is also possible that harmful chemicals in alder leaves, such as hirsutanol, might be involved in interfering with normal larvae development.

In contrast, the longevity of Erisan fed with leaves of white birch (*Betula platyphylla* var. *japonica*) was shorter because the level of defensive compounds in white birch is quite high, with large amounts of both condensed tannin and total phenolics. Higher levels of these compounds were found in seedlings grown in infertile soil and $[CO_2]^e$ (SHIBATA & al. 2004). In general, when plants are grown in infertile conditions, their level of defense chemicals are usually high, this pattern can be explained by the carbon-nutrient balance (CNB) hypothesis (BRYANT & al. 1983) and/or the nutrient availability hypothesis (COLEY & al. 1985). Furthermore, under a –N' treatment, defense chemicals in birch leaves tended to be higher at $[CO_2]^e$ than $[CO_2]^a$ (SHIBATA & al. 2004). At $[CO_2]^e$, excess assimilated CO_2 under a limited nutrient condition will result in more carbon-based defense chemicals in birch seedlings.

Under the fertile condition, no difference was found in the amount of total phenolics and condensed tannin in alder between CO_2 levels. However, under poor soil nutrient conditions, deciduous broadleaf trees tend to increase their defense capacity through higher amounts of condensed tannin and total phenolics (e.g. LINDROTH 1995). Indeed, longevity of larvae fed with leaves at $[CO_2]^e$-N was ca. 2.5 days longer than those fed with leaves at $[CO_2]^e$+N (Fig. 2). If the idea of CNB holds in alder, we would expect larval longevity to be shorter under $[CO_2]^e$-N, the opposite to what was found.

Why did mountain alder show a lower defense level in infertile soil in contrast to other species, such as birch (SHIBATA & al. 2004), poplar, maple (LINDROTH 1995), larch and beech (KOIKE & al. 2003)? One possible reason is that activities of the symbiotic *Frankia* sp. is accelerated by $[CO_2]^e$-N (Fig. 3). In fact, despite the large difference in N application between +N and –N, no difference in leaf nitrogen concentration was found in alder seedlings grown in $[CO_2]^e$-N and $[CO_2]^e$+N. These results imply that under increasing atmospheric CO_2, plants with nitrogen fixing capabilities such as mountain alder may express changes in defense traits mediated by the number and the activity of nodules formed by *Frankia* sp. in the root system. Nevertheless, the question why the slightly short survival time of Erisan fed with alder leaves at $[CO_2]^e$+N is still unclear. One possible explanation is the production of trichome was increased by $[CO_2]^e$+N.

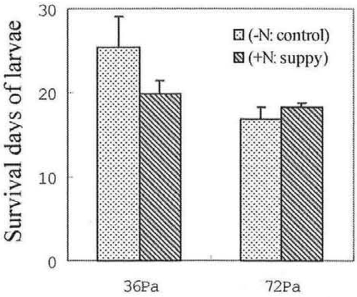

Fig. 1. Survival of larvae fed with alder leaves treated in the combination of CO_2 and nutrients. Statistical test of the treatment effects is : CO_2***, N-Supply***, CO_2 x N-Supply***, where *** is $p<0.001$

While plants grown under $[CO_2]^e$ often show discolouration of foliage (e.g., pale green leaves), especially under infertile soil condition (BAZZAZ 1990), in alder leaves, however, no significant differences in nitrogen and chlorophyll content was found between $[CO_2]$ ±N (data not shown). This indicates that under a carbon-rich environment, the enhanced activity of the symbiont of alder can compensate for the plant's deficiency in nitrogen. This study also found that the net photosynthetic rate measured at saturated light and CO_2 in alder seedlings treated with $[CO_2]^e$-N was slightly lower than those treated with $[CO_2]^a$+N (data not shown). Since carbon-based secondary chemicals originate from photosynthates, the assumption that

$[CO_2]^a$+N was the most endowed in defensive chemicals is supported by the shortest larval survival time in the bioassay. We conclude that although an accelerated activity of *Frankia* sp. in alder can mitigate a lack of plant nitrogen in $[CO_2]^e$–N plants, the cost in photosynthates to *Frankia* probably reduced the alder's carbon-based defense capacity.

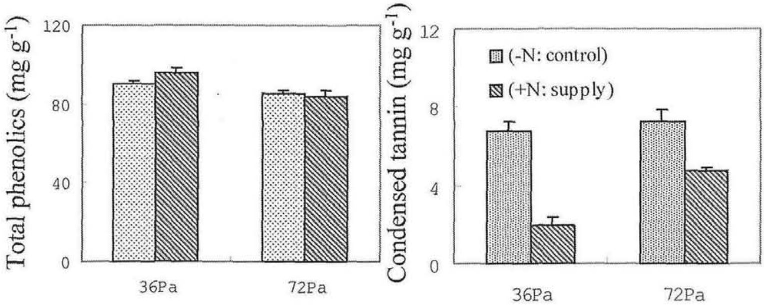

Fig. 2. Concentration of defense chemicals (total phenolics and condensed tannin) of alder leaves raised under two levels of CO_2 and nutrient. Statistical test of the treatment effects of total phenolics is: CO_2^{***}, N-Supply***, CO_2 x N-Supply***; that for condensed tannin is: CO_2^{***}, N-Supply ns, CO_2 x N-Supply*, where *** is $p<0.001$, * is $p<0.05$ and ns is not significant.

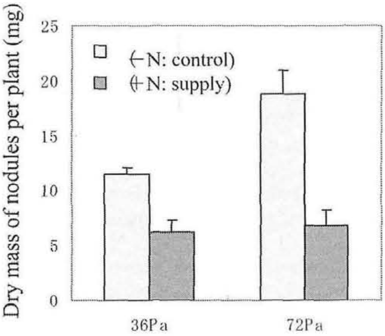

Fig. 3. Nodule formation of alder seedlings grown under the two levels of CO_2 and nutrient. Statistical test of the treatment effects of dry mass of nodules per plant is: CO_2***, N-Supply***, CO_2 x N-Supply*, where * is $p<0.05$ and *** is $p<0.001$.

Acknowledgements

Financial support was in part by the RR-2002 project of MEXT, Japan and JSPS (Basic research B, No. 14360078).

References

BAUR R., BINDER S. & BENZ G. 1991. Nonglandular leaf trichomes as short-term inducible defense of the grey alder, *Alnus incana* (L.), against the chrysomelid beetle, *Agelastica alni* L. - Oecologia 87: 219-226.

BAZZAZ F.A. 1990. The response of natural ecosystems to the rising global CO_2 levels. – Ann. Rev. Ecol. Syst. 21: 167-196.

BRYANT J. P., CHAPIN F. S. III & KLEIN D. R. 1983. Carbon / nutrient balance of boreal plant in relation to vertebrate herbivory. - Oikos 40: 357-368.

COLEY P. D., BRYANT J. P. & CHAPIN F. S. III 1985. Resource availability and plant antiherbivore defense. - Science 230: 895-899.

KOIKE T., IZUTA T., LEI T. T., KITAO M. & ASANUMA S. 1997. Effects of high CO_2 on nodule formation in roots of Japanese mountain alder seedlings grown under two nutrient levels. - Plant and Soil 195: 887-888.

— , MATSUKI S., MATSUMOTO T., YAMAJI K., TOBITA H., KITAO M. & MARUYAMA Y. 2003. Bottom-up regulation for protection and conservation of forest ecosystems in northern Japan under changing environment. - Eurasian J. For. Res. 6: 177-189.

LINDROTH R. L. 1995. CO_2-mediated changes in tree chemistry and tree-Lepidoptera interactions. - In: KOCH G. W. & MOONEY H. A. (Eds.), Carbon dioxide and terrestrial ecosystems, pp.105-120. - Academic Press. San Diego, U.S.A.

MATSUKI S., SANO Y. & KOIKE T. 2004. Chemical and physical defense in the early and late leaves in three heterophyllous birch species native to northern Japan. - Ann Bot. 93: 141-147.

SHIBATA T., TAKEUCHI Y., MATSUKI S., TOBITA H., KITAO M., MARUYAMA Y. & KOIKE T. 2004. Effects of elevated CO_2 on the leaf quality of birch and oak seedlings affecting survival of insect larvae in combination with two nutrient regimes. - Transaction of Hokkaido Br., Jpn. For. Soc. 52: 81-83.

Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 2005

Band/Volume: 45_4

Autor(en)/Author(s): Koike Takayoshi, Lei T. T., Shibata T., Matsuki S., Tobita H., Kitao M., Yamashita N., Quoreshi A. M., Maruyama Y.

Artikel/Article: Characteristics of Defense Chemicals of Mountain Alder Seedlings Raised under Elevated CO2 and Nitrogen Supply. 163-168