Responses of Ectomycorrhizal Colonization and Morphotype Assemblage of *Quercus myrsinaefolia* Seedlings to Elevated Air Temperature and Elevated Atmospheric CO₂

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Ectomycorrhizal colonization and morphotype assemblage of *Quercus myrsinaefolia* seedlings were compared under ambient (399 $\mu\text{l liter}^{-1}$ of CO₂) atmospheric, elevated air temperature (ca. +4.5°C) (ET), and elevated air temperature (ca. +4.5°C) plus elevated atmospheric CO₂ (753 $\mu\text{l liter}^{-1}$) (ET-EC) conditions in Experiment 1. In Experiment 2, the ectomycorrhizal status was compared under ambient (395 $\mu\text{l liter}^{-1}$ of CO₂) and elevated CO₂ (488 $\mu\text{l liter}^{-1}$) (EC) conditions. The total ectomycorrhizal colonization and the dry weight of the plants significantly decreased under the ET and ET-EC conditions, indicating that the elevated temperature must be above the optimum for ectomycorrhizal colonization. Three ectomycorrhizal morphotypes, *Cenococcum geophilum* type, Type 12 and Type 40, showed relatively higher colonization under the ambient condition. Colonization of Type 12 significantly decreased, and that of Type 40 was completely prevented by the ET and ET-EC treatments, suggesting that colonization of these two morphotypes is sensitive to the elevated temperature. The ergosterol content in the fine roots significantly increased under the EC condition, suggesting that the ectomycorrhizal fungal mass was increased by CO₂ enrichment.

Key words: ectomycorrhiza, temperature, CO₂, *Quercus myrsinaefolia*

The atmospheric CO₂ concentration has increased rapidly since the industrial revolution due to increasing human activities. The increases of atmospheric CO₂ concentration and other greenhouse gases may cause global warming¹⁶. Evaluation of the response of vegetation-soil systems to elevated air temperature and elevated atmospheric CO₂ concentration is one of the most important issues concerning the global environment today because this system has two func-

tions, i.e., as a CO₂ sink (plant assimilation) and a CO₂ source (soil and plant respiration). Many studies have shown that the elevation of atmospheric CO₂ enhances the photosynthetic rate and plant growth under suitable nutrient conditions⁴. However, these positive effects of elevated CO₂ may be restricted by water and nutrient availability⁹.

It has been suggested by numerous researchers that mycorrhizae confer benefits on host plants by enhancing water and nutrient availability, and play important roles in the carbon cycle in terrestrial ecosys-

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tems²⁾. Ectomycorrhizae are suggested to invest additional carbon in specialized structures, such as fruit bodies, rhizomorphs and extensive extraradical mycelia. They have higher carbon sink strength than do endophytic mycorrhizae²¹⁾.

There have been many conflicting reports about the responses of ectomycorrhizae of trees exposed to elevated CO₂. Some showed an increase (e.g., *Pinus echinata* and *Quercus alba*²²⁾; *Betula papyrifera* and *Pinus strobus*¹¹⁾; *Pinus palustris*³¹⁾) and others did not show an increase (e.g., *Pinus taeda*¹⁸⁾; *Tsuga canadensis*¹¹⁾) in ectomycorrhizal colonization with CO₂ enrichment. It has been suggested that there are large variations in the productivity of the extraradical mycelium⁶⁾ and the ability to improve nutrient status^{1,9,30)} among ectomycorrhizal fungi. There have been few studies on interactive effects of elevated atmospheric CO₂ and elevated air temperature on ectomycorrhizal colonization and assemblage of ectomycorrhizal fungi, despite predictions that the average global atmospheric CO₂ and air temperature may both increase in the future.

Here, we show the responses of ectomycorrhizal

colonization and morphotype assemblage of *Quercus myrsinaefolia* (evergreen oak) seedlings exposed to elevated air temperature and elevated atmospheric CO₂ concentration.

Q. myrsinaefolia is distributed in Eastern and Southeastern Asia, and forests dominated by this plant are found in the Kanto region of eastern Japan¹⁰⁾. The Northern limit of the distribution of these forests is around the Northern Kanto region, while the Northern limit of the species distribution is around the coastland in southern Miyagi Pref., northeastern Japan. The examination of the effects of elevated air temperature and elevated atmospheric CO₂ on ectomycorrhizae associated with this plant grown near the northern limit of its territory would give valuable information for evaluating the effects of global warming on the survival and movement of ectomycorrhizal fungal species.

Materials and Methods

Experiment 1

The ectomycorrhizal status of *Q. myrsinaefolia*

TGC (Temperature Gradient Chamber)

CTGC (CO₂-Temperature Gradient Chamber)

Fig. 1. Chambers used in Experiment 1.

seedlings was compared using Ambient, elevated air temperature (ET), and elevated air temperature plus elevated atmospheric CO₂ concentration (ET-EC) treatments. These treatments were set up in a Temperature Gradient Chamber (TGC) and a CO₂-Temperature Gradient Chamber (CTGC) (Fig. 1; Lee and Oikawa, unpublished). These chambers were set in the Environmental Research Center (36°5'N, 140°5'E) at the University of Tsukuba, Tsukuba, Ibaraki, eastern Japan, which is located near the northern limit of *Q. myrsinaefolia* forests. The annual mean air temperature, annual precipitation, and Kira's warmth and coldness indices during 1981 to 1994 in the Center were 13.4°C, 1255 mm, 105.0°Cmonth and -5.5°Cmonth, respectively. The TGC is a 30-m-long greenhouse. There are electric fans on one side that create continuous airflow; solar radiation heats the air in the chamber, resulting in a temperature gradient from the entrance to the exit. In cloudy weather and at night, heated air is additionally supplied from holes in plastic pipes attached from the entrance to the exit on both sides inside the chamber to maintain the temperature gradient. The airflow rate (0.1–0.8 m sec⁻¹) is controlled to maintain a 5°C difference between the entrance and a point 25 m inside. The photosynthetic rate has been found to be highest at airflow rates between 0.5–1.0 m sec⁻¹ (38). The airflow rate in the TGC is often in this range in the daytime. The air conditions at the entrance are the same as the Ambient conditions. In the CTGC, 100% CO₂ gas is emitted from holes in plastic pipes attached inside the chamber. Continuous airflow creates a gradient of CO₂ concentration as well as a temperature gradient. The CO₂ concentration at a point 25 m inside the chamber is maintained at twice the Ambient concentration. Neither the heated air nor the CO₂ gas blow directly on the plants. The monthly mean air temperature and CO₂ concentration during the experimental period are shown in Fig. 2. The mean air temperatures for the Ambient, ET and ET-EC treatments during the experiment were 21.3°C, 25.8°C and 26.0°C, respectively. The means ± standard deviations of the CO₂ concentration for the Ambient and ET-EC treatments during the experiment were 399 ± 41 µl liter⁻¹ and 753 ± 129 µl liter⁻¹, respectively.

Fig. 2. The monthly mean air temperature (a) and atmospheric CO₂ concentration (b) in Experiment 1.

The mean air temperatures with the Ambient (○), elevated temp (ET; □), and elevated temp and elevated CO₂ (ET-EC; △) treatments during the whole period were 21.3°C, 25.8°C and 26.0°C, respectively. The means ± standard deviations of CO₂ for the Ambient (○) and ET-EC (△) treatments during the whole period were 399 ± 41 µl liter⁻¹ and 753 ± 129 µl liter⁻¹, respectively. The error bars indicate standard deviations.

ly. Although the CO₂ concentration during the ET treatment was not monitored continuously, occasional measurements showed the same CO₂ concentration as for the Ambient treatment in the daytime, and a slightly higher value at night. The means ± standard deviations of relative humidity and air vapour pressure deficit (VPD) for the Ambient, ET and ET-EC treatments during the experiment were 89.9 ± 5.5%, 80.8 ± 3.8% and 75.3 ± 6.2%, and 3.0 ± 2.6 Pa kPa⁻¹, 7.4 ± 3.1 Pa kPa⁻¹ and 9.4 ± 5.0 Pa kPa⁻¹, respectively. The photosynthetic rate is hardly affected, and transpiration rate is positively correlated with VPD when VPD is below 10 Pa kPa⁻¹ (3,25,36). The relative photosynthetic photon flux density was about 85% in both the chambers.

Soil from the habitat of *Q. myrsinaefolia* was used as the inoculum of ectomycorrhizae. Blocks of 12 cm × 12 cm × 5 cm (depth) of surface mineral soil were collected at 15 points in a *Q. myrsinaefolia* stand in the

University of Tsukuba on 7 May 1997. The canopy of the stand was composed of only *Q. myrsinaefolia*. The soil blocks were sieved through a 2-mm mesh to eliminate stones. Root fragments trapped on the mesh were added to the sieved soil. The soil was mixed well and used for the experiment.

Seeds of *Q. myrsinaefolia* were purchased from Kawakami Nojo Co., Ltd., Ibaraki, Japan, and stored in wet sand at ca. 4°C. The seeds were washed with tap water, surface-sterilized with 70% ethanol for 2 min, and then rinsed 3 times with sterilized distilled water (SDW). The seeds were germinated in the laboratory in vermiculite which had been autoclaved for 1 h, with adequate addition of SDW. After about a month, each seedling of which the first foliage leaf had begun to unfold was transplanted into a set of sterile square petri dishes (14 cm × 9 cm × 1.5 cm) with a 130 g wet weight of the soil. A hole of about 1 cm × 1 cm was made in a side of the petri dish, and the shoot was placed so that it was sticking through it. After addition of 30 ml of SDW, each petri dish was sealed with parafilm. The petri dishes were wrapped with aluminum foil and set vertically. The seedlings were grown in the laboratory for a week and then grown under each treatment condition from the 17th of May until the 29th of September 1997 (for about 19 weeks). SDW was supplied by a syringe every 1 to 2 weeks to compensate for losses of the water content of the collected soil.

After the experiment, 9 to 10 seedlings per treatment were harvested. Five of these per treatment were used to examine ectomycorrhizal colonization and morphotypes. Each seedling was separated into leaves, stem and root, and the leaves and stem were oven-dried at 80°C and weighed. Carbon and nitrogen contents of the leaves were determined with an NC analyzer (Sumigraph NC-900, Sumika Chemical Analysis Service, Ltd., Osaka). The root was thoroughly washed with tap water on a 0.5-mm mesh. Five to 7 first-order lateral roots per seedling were picked off and examined for ectomycorrhizal colonization and morphotypes. The tips (range: 255–1893 pieces) of these roots were observed with a dissecting microscope, and ectomycorrhizae were classified into morphotypes based on color and morphological

characteristics according to Ingleby *et al.*¹⁵⁾ and Yamada and Katsuya³⁹⁾. These tips of each morphotype were examined to confirm the morphological typing using Nomarski interference microscopy. The total ectomycorrhizal colonization was calculated as the ratio of the number of ectomycorrhizal root tips to the total number of root tips examined. Colonization of each morphotype was also calculated in the same way.

It has been stated that ectomycorrhizal mass should be estimated in studies on the response of ectomycorrhizae to atmospheric change^{5,13)}. Ergosterol, which is a prominent sterol of fungal cell membranes³⁷⁾ and an indicator of vital fungal mass¹⁹⁾, was quantified to estimate ectomycorrhizal fungal mass in roots. The remaining harvested seedlings (4 or 5 from each treatment) were separated into leaves, stem and root. The dry weights of the leaves and stem were determined. Carbon and nitrogen contents of the leaves were determined with the NC analyzer. After washing thoroughly, fine roots (≤ 1 mm in diameter) were separated from the whole root, and the dry weight of the remaining thicker roots was determined. The fine roots were frozen at -80°C , freeze-dried, weighed, ground with a mortar and pestle, and used to determine ergosterol content. Ergosterol was quantified according to Kasai and Horikoshi¹⁷⁾. The carbon content in an aliquot of the fine root particles was determined with the NC analyzer. The ergosterol content was expressed relative to the fine root carbon content.

The dry weight of randomly sampled seedlings at the time of the initial transplantation in the soil was 0.11 ± 0.05 g (mean $\pm$ S.D., $n=4$). No ectomycorrhizal colonization was found, and ergosterol was not detected in the roots of these seedlings.

Experiment 2

The ectomycorrhizal status of *Q. myrsinaefolia* seedlings was compared between the Ambient and elevated atmospheric CO₂ concentration (EC) treatments. Two open-top chambers for the Ambient and EC treatments were built in a field near the Environmental Research Center at the University of Tsukuba (Ishikawa, unpublished). The chambers had 1 m × 1 m

×1 m (height) dimensions, and the sides of the chambers were made of transparent plastic sheets. The chambers were covered with black, coarse-textured plastic sheets to prevent excessive heating by the sun. The elevated CO₂ condition was established using CO₂ emission from litter decomposition. The monthly mean air temperatures and CO₂ concentrations in the treatments are shown in Fig. 3. The mean air temperatures of the Ambient and EC treatments during the experiment were 26.0°C and 25.9°C, respectively. The means ± standard deviations of the CO₂ concentration of the Ambient and EC treatments during the experiment were 395 ± 48 µl liter⁻¹ and 488 ± 64 µl liter⁻¹, respectively. The relative photosynthetic photon flux density was about 60% in both the chambers.

Blocks of 10 cm × 10 cm × 5 cm (depth) of surface mineral soil were collected at 10 points in the same stand used in Experiment 1 on 2 July 1997. Preparations of soil and seedlings were the same as in Experiment 1. The seedlings were grown from the 8th of July until the 14th of October 1997 (for about 15 weeks) for each treatment. The seedlings were har-

Fig. 3. The monthly mean air temperature (a) and atmospheric CO₂ concentration (b) in Experiment 2.

The mean air temperatures with the Ambient (○) and an elevated CO₂ (EC; ●) treatments during the whole period were 26.0°C and 25.9°C, respectively. The means ± standard deviations of CO₂ for the Ambient (○) and EC (●) treatments during the whole period were 395 ± 48 µl liter⁻¹ and 488 ± 64 µl liter⁻¹, respectively. The error bars indicate standard deviations.

Table 1. Variables^a examined in Experiment 1.

	Ambient	ET ^b	ET-EC ^c	P ^d
Dry weight of shoot (g)	1.19 ± 0.18* (n=5)	0.62 ± 0.27† (n=5)	0.63 ± 0.12† (n=5)	< 0.005
Ectomycorrhizal colonization	0.60 ± 0.05* (n=5)	0.15 ± 0.16† (n=5)	0.10 ± 0.17† (n=5)	< 0.005
Dry weight of whole plant (g)	2.18 ± 0.25* (n=5)	0.94 ± 0.30† (n=5)	1.03 ± 0.19† (n=4)	< 0.001
Root/shoot ratio	0.82 ± 0.12* (n=5)	0.55 ± 0.06† (n=5)	0.48 ± 0.12† (n=4)	< 0.01
Ergosterol content in fine roots (µg g ⁻¹ root C)	0.54 ± 0.16* (n=5)	0.27 ± 0.27* (n=5)	0.25 ± 0.16* (n=4)	= 0.090
Carbon content of leaves (%)	46.1 ± 0.7* (n=10)	46.5 ± 0.7* (n=10)	46.5 ± 0.7* (n=9)	= 0.441
Nitrogen content of leaves (%)	1.64 ± 0.22* (n=10)	2.36 ± 0.30† (n=10)	2.58 ± 0.26† (n=9)	< 0.0001
C/N ratio of leaves	28.6 ± 3.9* (n=10)	20.0 ± 2.5† (n=10)	18.2 ± 1.9† (n=9)	< 0.0001

^a Mean ± standard deviation; values followed by different marks in each row are significantly different among the three treatments.

^b Elevated temperature.

^c Elevated temperature and elevated CO₂.

^d Significant level by ANOVA followed by Scheffe's test for multiple comparisons.

vested and examined for the same variables as in Experiment 1.

Statistical analysis

Analysis of Variance (ANOVA) was carried out to analyze the variables. Scheffe's test and the *t*-test were applied for multiple comparisons and for binary comparisons, respectively. Mann-Whitney's *U*-test was applied to the data of which frequency distributions were not normal. The difference of colonization of each morphotype among the treatments was analyzed by the Kruskal-Wallis test followed by Mann-Whitney's *U*-test for multiple tests. All the statistical analyses were made using Stat View version 4.5²⁸⁾.

Results

Experiment 1

Table 1 shows the variables examined in Experiment 1. The dry weight of shoots was significantly decreased (by 48%) by the ET treatment and decreased by 47% by the ET-EC treatment compared with the Ambient treatment (Scheffe's test, $P < 0.005$). The ectomycorrhizal colonization was significantly decreased (by 74%) by the ET treatment and decreased by 83% by the ET-EC treatment compared with the Ambient treatment (Scheffe's test, $P < 0.005$). The dry weight of the whole plant was significantly decreased (by 57%) by the ET treatment and decreased by 53% by the ET-EC treatment compared with the Ambient treatment (Scheffe's test, $P < 0.001$). The root/shoot ratio was significantly decreased by the ET and ET-EC treatments (Scheffe's test, $P < 0.01$). The average ergosterol content in fine roots tended to be decreased by the ET and ET-EC treatments, but the differences among the three treatments were not significant (ANOVA, $P = 0.090$). This appeared to be due to the high coefficients of variation for the ET (99.3%) and ET-EC (64.9%) treatments. The carbon content of leaves was not significantly different among the three treatments (ANOVA, $P = 0.441$). The nitrogen content of leaves was significantly higher with the ET and ET-EC treatments than with the Ambient treatment (Scheffe's test, $P < 0.0001$). The C/N ratio

was significantly lower with the ET and ET-EC treatments than with the Ambient treatment (Scheffe's test, $P < 0.0001$).

Fig. 4 shows the colonization of morphotypes for the three treatments in Experiment 1. Altogether, 7 morphotypes were found. Three morphotypes, Cg, Type 12 and Type 40, showed relatively higher colonization (average colonization ≥ 0.01) with the Ambient treatment. Cg indicates *Cenococcum geophilum* type, identified by a black color, a sheath surface of net synenchyma or regular synenchyma, a star-like arrangement of surface cells of the sheath and lack of clamped septa¹⁵⁾. The numbers of the other morphotypes were given by the first author for convenience sake. Type 12 has a white color, a sheath surface of net prosenchyma and lack of clamped septa. Type 40 has a brown color, a sheath surface of irregular synenchyma and clamped septa. Characteristics of the other minor morphotypes were as follows. Type 2 has a white color, a sheath surface of net synenchyma, numerous emanating hyphae and clamped septa. Type 45 has a white color, a sheath surface of net prosenchyma, acicular or hypha-like cystidia and clamped septa. Type 46 has a white color, a sheath surface of net prosenchyma, acicular cystidia and lack of clamped septa. Type 47 has a white color, a sheath surface of irregular synenchyma, capitate cystidia and clamped septa.

The response to the elevated temperature was found to be different among the three major ectomycorrhizal morphotypes described above (Fig. 4). Cg showed the same colonization level with the ET and ET-EC treatments as with the Ambient treatment (Kruskal-Wallis test, $P = 0.3946$). The colonization of Type 12 was significantly lower with the ET and ET-EC treatments than with the Ambient treatment (Kruskal-Wallis test, $P < 0.05$ for family-wise; Mann-Whitney's *U*-test, $P < 0.02$ for comparison-wise). Type 40 was not found with the ET and ET-EC treatments, as determined by analyzing the number of seedlings on which Type 40 colonized, by Fisher's exact probability test ($P < 0.05$). Elevated CO₂ had no significant effect on the morphotype colonization, as determined by comparing the ET-EC treatment to the ET treatment (Mann-Whitney's *U*-test, $P \geq 0.10$).

Fig. 4. The colonization of ectomycorrhizal morphotypes in Experiment 1.

ET, elevated temp; ET-EC, elevated temp and elevated CO₂; Cg, *Cenococcum geophilum* type; Non, non-colonization. The numbers in parentheses indicate (no. of seedlings on which the morphotype colonized)/(no. of seedlings examined). The bars with different marks in each morphotype are significantly different among the three treatments by the Kruskal-Wallis test ($P < 0.05$) followed by Mann-Whitney's U -test ($P < 0.02$) in Cg and Type 12, and by Fisher's exact probability test ($P < 0.05$) in Type 40. The 's' mark implies average colonization of less than 0.01. The error bars indicate standard deviations.

Experiment 2

Table 2 shows the variables examined in Experiment 2. There were no significant differences between the Ambient and EC treatments in the dry weight of shoot (t -test, $P = 0.917$) and the total ectomycorrhizal colonization (t -test, $P = 0.944$). The average dry weight of the whole plant was slightly higher with the EC treatment than with the Ambient treatment, but the difference was not significant (t -test, $P = 0.183$). The root/shoot ratio did not differ significantly between the two treatments (t -test, $P = 0.404$). The ergosterol content in fine roots with the EC treatment was significantly higher (by 26%) than that with the Ambient treatment (Mann-Whitney's U -test, $P < 0.05$). There were no significant differences between the two treatments in contents of carbon (t -test, $P = 0.169$) and nitrogen (t -test, $P = 0.197$), or the C/N ratio (t -test, $P = 0.232$) of the leaves.

There was no significant difference in colonization of any of the morphotypes between the Ambient and EC treatments (Fig. 5; Mann-Whitney's U -test, $P > 0.10$).

Discussion

Elevated temperature suppressed ectomycorrhizal colonization and plant growth of *Q. myrsinaefolia* seedlings (see Table 1). Theodorou and Bowen³⁴ reported that the optimal temperature for ectomycorrhizal formation on *Pinus radiata* by four fungal isolates was 25°C. The maximum formation of ectomycorrhizae on *Pseudotsuga menziesii* and *Pinus ponderosa* occurred between 18.5–24°C in the soil, and their formation was greatly reduced or prevented at or above 29.5°C²³. In the ET and ET-EC treatments in Experiment 1, the mean air temperature was above 30°C in the daytime in late May, and the mean monthly temperatures were above 26°C from June to September. Thus, the elevated temperature in this study must have been above the optimum for ectomycorrhizal colonization during most of the period of this experiment. This may be the reason for the lack of a positive effect of elevated CO₂ on ectomycorrhizal colonization in the ET-EC treatment compared to the ET treatment.

The suppression of ectomycorrhizal colonization

Table 2. Variables^a examined in Experiment 2.

	Ambient	EC ^b	P ^c
Dry weight of shoot (g)	0.88 ± 0.02* (n=4)	0.86 ± 0.07* (n=4)	=0.917
Ectomycorrhizal colonization	0.71 ± 0.15* (n=4)	0.70 ± 0.13* (n=4)	=0.944
Dry weight of whole plant (g)	1.16 ± 0.05* (n=4)	1.58 ± 0.28* (n=4)	=0.183
Root/shoot ratio	0.55 ± 0.01* (n=4)	0.49 ± 0.01* (n=4)	=0.404
Ergosterol content in fine roots (μg g ⁻¹ root C)	0.64 ± 0.03* (n=4)	0.80 ± 0.12† (n=4)	<0.05
Carbon content of leaves (%)	44.7 ± 0.3* (n=8)	45.1 ± 0.2* (n=8)	=0.169
Nitrogen content of leaves (%)	1.68 ± 0.04* (n=8)	1.80 ± 0.03* (n=8)	=0.197
C/N ratio of leaves	27.0 ± 10.9* (n=8)	25.2 ± 5.2* (n=8)	=0.233

^a Mean ± standard deviation; values followed by different marks in each row are significantly different between the two treatments.

^b Elevated CO₂.

^c Significant level by the *t*-test except for ergosterol content in fine roots which was tested by the Mann-Whitney's *U*-test.

may cause suppression of subsequent plant growth, especially root growth. Mycorrhizae stimulate host plants to allocate more carbon below the ground³²⁾. Rousseau and Reid²⁹⁾ reported that high levels of ectomycorrhizal colonization increased the carbon sink strength, which was responsible for the enhancement of CO₂ assimilation. The suppression of CO₂ assimilation is suggested to account for the lower C/N ratio of the leaves in the ET and ET-EC treatments.

The response of three-year-old saplings of *Q. myrsinaefolia* to the elevated air temperature and elevated CO₂ was also simultaneously investigated with the Ambient, ET and ET-EC treatments (Usami and Oikawa, unpublished). Each sapling was grown in a pot (diameter of 15 cm, depth of 30 cm), with soil artificially prepared from commercially obtained materials, and supplied with adequate nutrients and water, from Feb. 1997 until March 1998. The relative growth rate (mg g⁻¹ day⁻¹) and net assimilation rate (g m⁻¹ day⁻¹) was highest with the ET-EC treatment and lowest with the Ambient treatment from March until May. The dry weight of the saplings was higher by 49% with the ET treatment and by 140% with the ET-EC treatment compared with the Ambient treat-

Fig. 5. The colonization of ectomycorrhizal morphotypes in Experiment 2.

EC, elevated CO₂; Cg, *Cenococcum geophilum* type; Non, non-colonization. The numbers in parentheses indicate (no. of seedlings on which the morphotype colonized)/(no. of seedlings examined). The bars with the same mark in each morphotype are not significantly different between the two treatments by the Mann-Whitney's *U*-test ($P > 0.10$). The 's' mark implies average colonization of less than 0.01. The error bars indicate standard deviations.

ment after 14 months. Ectomycorrhizal colonization was observed qualitatively in these saplings at the start of the experiment. Seedlings that were non-ectomycorrhizal at the beginning were used in the present study, and they responded negatively with the ET and ET-EC treatments. Establishment of ectomycorrhizae before the early stage of the growing season of the plant may support resistance to elevated air temperature and a positive response to elevated CO₂.

The elevated CO₂ did not increase ectomycorrhizal colonization (ratio of the number of ectomycorrhizal root tips to the total number of root tips examined), but increased the ectomycorrhizal fungal mass with the Ambient temperature in Experiment 2 (see Table 2). This suggests that the elevated CO₂ increased the mycorrhizal length and/or development of fungal tissues in the root. The *Pinus* species has frequently been used in previous studies on responses of ectomycorrhizae to elevated CO₂^{11,14,22,31}). The level of colonization was evaluated as the percentage of mycorrhizal root tips among the total root tips, or the number of mycorrhizal root tips or clusters in those studies. Ectomycorrhizae form on the lateral short roots of conifers such as the *Pinus* species, and then dichotomously branch and develop coralloid clusters⁹). Therefore, the number of mycorrhizal root tips of conifers may represent the approximate ectomycorrhizal mass. On the other hand, the roots of *Q. myrsinaefolia*, a broad-leaved tree, develop a racemose system of branches, and ectomycorrhizae of various lengths develop. Thus, the number of apices would not be suitable for representing the ectomycorrhizal mass. Ergosterol would be more useful for estimating the ectomycorrhizal fungal mass in the roots of broad-leaved trees.

Excessive photosynthates produced due to the elevated CO₂ may have been drained into the ectomycorrhizal fungal mass in the EC treatment. An increase of root dry weight has been reported in many plant species under elevated CO₂²⁷). Fine roots, which are the major sites of mycorrhizal colonization, are more responsive to CO₂ than coarse roots^{7,20}). It has been demonstrated that ectomycorrhizal plants provide substantial carbohydrate to mycobio-

nts^{8,26}). Colpaert *et al.*¹⁵) reported a negative correlation between the development of the fungal tissues, including the extraradical mycelium, of ectomycorrhizae and the growth of host plants. This suggested that the reduction in the plant growth was due to an energy drain by the fungi. There were no significant increases in the dry weight of whole plant and the root/shoot ratio, while the ergosterol content in fine roots significantly increased, in the EC treatment (see Table 2).

The elevated temperature reduced the colonization of Type 12 and prevented that of Type 40 (Fig. 4). Colonization of these morphotypes must be sensitive to the elevated temperature. The colonization of Type 12 was highest among the morphotypes in the Ambient treatment. So, decreases of the total ectomycorrhizal colonization were almost completely due to the reduction of colonization of Type 12. Colonization of Type 40 was found in 3 of the 5 seedlings examined in the Ambient treatment in Experiment 1, and was not found in Experiment 2. This suggests that the density of the inoculum of Type 40 was relatively lower, or its distribution was partial in the soil. Nevertheless, Type 40 must be most sensitive to the elevated temperature among the morphotypes because of its disappearance with the ET and ET-EC treatments. The *C. geophilum* type (Cg) showed the same colonization level with the ET and ET-EC treatments as with the Ambient treatment (Fig. 4). It has been recognized that *C. geophilum* has a wide mycorrhizal host range and a relatively wide geographical distribution³⁵). The greater drought tolerance of *C. geophilum* relative to other mycorrhizal fungi has been demonstrated experimentally³³). Survival of the mycorrhiza formed by *C. geophilum* was reported in a dry field²⁴). *C. geophilum* can thus survive and form mycorrhiza under environmental stresses such as the elevated temperature in this study.

We did not examine the responses of the extraradical mycelia of ectomycorrhizal fungi to the treatments in this study because of the difficulty in estimation of these in unsterilized soils derived from fields. Ineichen *et al.*¹⁴) reported that higher CO₂ levels increased extraradical mycelium of *Pisolithus tinctorius* on *Pinus silvestris* by a monoxenic cul-

ture. A study of the responses of the extraradical mycelium to elevated air temperature and elevated atmospheric CO₂ will be required using isolates of *C. geophilum*, Type 12 and Type 40 in the future.

Further research on the responses of ectomycorrhizae to atmospheric change using less disturbed soil will be required. The numbers of ectomycorrhizal morphotypes found in Experiments 1 and 2 were 7 and 5, respectively, which is considerably lower than the 25 and 20 found on the roots in the soil blocks which were concurrently collected at points adjacent to the blocks used for the experiments (Kasai, unpublished data). Mixed soil was used to reduce the heterogeneity of the inocula for these comparative experiments. The disturbance of soil may cause substantial selection of morphotypes for colonization.

This study screened morphotypes sensitive to elevated temperature, such as Type 12 and Type 40, and provides estimates of their likely fluctuations in the field due to global climate change.

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