Effects of Elevated Atmospheric CO₂ Concentration on the Infection of Rice Blast and Sheath Blight

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ABSTRACT

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The effect of elevated atmospheric CO₂ concentration on rice blast and sheath blight disease severity was studied in the field in northern Japan for 3 years. With free-air CO₂ enrichment (FACE), rice plants were grown in ambient and elevated (≈ 200 to $280 \mu\text{mol mol}^{-1}$ above ambient) CO₂ concentrations, and were artificially inoculated with consist of *Magnaporthe oryzae*. Rice plants grown in an elevated CO₂ concentration were more susceptible to leaf blast than those in ambient CO₂ as indicated by the increased number of leaf blast lesions. Plants grown under elevated CO₂ concentration had lower leaf silicon content, which may have contributed to the increased susceptibility to leaf blast under elevated CO₂

concentrations. In contrast to leaf blast, panicle blast severity was unchanged by the CO₂ enrichment under artificial inoculation, whereas it was slightly but significantly higher under elevated CO₂ concentrations in a spontaneous rice blast epidemic. For naturally occurring epidemics of the sheath blight development in rice plants, the percentage of diseased plants was higher under elevated as opposed to ambient CO₂ concentrations. However, the average height of lesions above the soil surface was similar between the treatments. One hypothesis is that the higher number of tillers observed under elevated CO₂ concentrations may have increased the chance for fungal sclerotia to adhere to the leaf sheath at the water surface. Consequently, the potential risks for infection of leaf blast and epidemics of sheath blight would increase in rice grown under elevated CO₂ concentration.

Additional keywords: *Pyricularia oryzae*, *Rhizoctonia solani*.

Atmospheric concentration of CO₂ has been increasing rapidly and is predicted to reach twice the preindustrial level during the later half of this century (9). Against the increasing demand for food by a growing human population, it is important to predict the effect of elevated concentration of CO₂ ([CO₂]_a) on global crop production.

Elevated [CO₂]_a is expected to increase dry matter production and yields in C₃ plants due to enhanced carbon fixation (18). Rice plants (*Oryza sativa* L.) increase the number of tillers in response to enriched [CO₂]_a under controlled environments, and have greater leaf area per plant with little changes in area of individual leaves (7). The increased number of tillers would produce more panicles and a higher grain yield (45). In contrast to the numerous reports about the effects of elevated [CO₂]_a on growth and yield of plants, there are few studies on the changes in plant disease progression in response to higher [CO₂]_a.

In the literature, it is not clear whether the disease severity is enhanced or diminished by a higher [CO₂]_a (4–6,13,26,37,38). Tomato plants showed increased tolerance to infection by *Phytophthora parasitica* Dastur under elevated ($700 \mu\text{mol mol}^{-1}$) [CO₂]_a conditions (12). Hibberd et al. (4–6) showed that [CO₂]_a at $700 \mu\text{mol mol}^{-1}$ reduced primary penetration of *Erysiphe graminis* de Candolle in barley leaves. Thompson et al. (37) and Thompson and Drake (38) reported that elevated [CO₂]_a reduced powdery mildew (*E. graminis*) on wheat and the severity of rust (*Puccinia sparganioides* Ellis & Barth) on C₃ sedge, *Scirpus olneyi* Grey.

However, the severity of infection with an unidentified fungal pathogen was increased in the C₄ grass, *Spartina patens* (Ait.) Muhl. Manning and Tiedemann (26) suggested that elevated [CO₂]_a would increase the plant size and canopy density, consequently, making the canopy microclimate more conducive to development of several diseases.

Rice blast is caused by *Pyricularia oryzae* Cavara (teleomorph *Magnaporthe oryzae* Couch and Kohn) and occurs in most rice-growing areas in the world (2,30). The disease spreads by airborne conidia produced in the lesions. With an extended period of surface wetness, the conidia land on a leaf surface, germinate, and penetrate leaf and other plant tissues following a suitable dew period, during which plant surfaces remain moist, to cause an infection. This disease is a typical polycyclic disease because many cycles of infection occur within a growing season (40). The blast pathosystem has two major subsystems, the leaf blast and the panicle blast. During the early growth stages of the host, lesions are formed mainly on leaves; whereas, after heading, the pathogen infects the panicles. Panicle blast causes direct yield losses, because grain filling is retarded. In Japan, the inoculum leading to panicle blast results from the conidia formed on the upper leaves blast lesions. Therefore, it is more important to control upper leaves' blast.

Sheath blight caused by *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) is also among the most widespread and persistent diseases of rice. The fungus over-seasons in the soil, mainly as sclerotia (20), which float on the water when rice paddies are flooded at the beginning of a rice season. Then, they attach themselves to a rice leaf sheath at the water line, germinate, and infect the rice plants. Therefore, the lesions first appear on the leaf sheath near the waterline. The

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disease is classified as the monocyclic disease (40). The disease progression in each season includes two phases: horizontal spreading and upward developing. As the rice canopy grows denser, the pathogen spreads from the infected tillers to adjacent plants via runner hyphae from the lesions. This phase is called the horizontal spreading phase, and affects disease incidence. On the other hand, the upward developing phase begins at the booting stage of rice plants. The disease spreads to the upper part of the plants by the runner hyphae.

With respect to the effects of elevated $[\text{CO}_2]_a$ on plants, many studies have used enclosures such as growth chambers and cabinets, which alter microclimate for the plants and may change the plant responses to elevated $[\text{CO}_2]_a$ (27). Indeed, Thompson and Drake (38) suggested a possibility that the disease severity of rust in the *Scirpus olneyi* plants inside the chambers was lower than that in the open air plots due to the barrier presented by the chambers. With rice blast and sheath blight, enclosures may affect disease development by altering the dispersion of the pathogens or the plant susceptibility to these diseases. The free-air CO_2 enrichment (FACE) technique has been used to grow plants under elevated $[\text{CO}_2]_a$ in the field without any enclosures (19). The technique could minimize the artificial effect that is inevitable with the enclosure approach. The Japanese Rice FACE, the world's first FACE with rice, was established with the core objective of investigating the effects of elevated $[\text{CO}_2]_a$ on rice growth and yield as well as ecosystem processes, including fungal pathogen infection (16,29). Rice crops were grown under two mole fractions of CO_2 (ambient and ambient plus $200 \mu\text{mol mol}^{-1}$)

(29). Elevated $[\text{CO}_2]_a$ increased nitrogen (N) uptake, dry matter, spikelet number, and grain yield when sufficient N was supplied over the whole season (16).

The overall objective of this study was to assess the effect of elevated $[\text{CO}_2]_a$ on the infection of the major rice diseases. Currently, no research exists on the effects of $[\text{CO}_2]_a$ on the development of rice diseases despite the energy dependence of the world's population on this crop. Among rice diseases, blast and sheath blight are the most serious and prevalent; hence, we focused and report on the effects of elevated $[\text{CO}_2]_a$ on the infection of these diseases in the field.

MATERIALS AND METHODS

Field experiments. A full description of the Rice FACE facility is provided by Okada et al. (29). The experiments were conducted in Shizukuishi, Iwate Prefecture in northern Japan over a 3-year period, from 1998 to 2000. The latitude, longitude, and altitude of the experimental site is $39^\circ 40' \text{N}$, 141°E , and $\approx 200 \text{ m}$, respectively.

The experiments were a completely randomized block design with two mole fraction of CO_2 replicated four times. Each block consisted of an elevated $[\text{CO}_2]_a$ plot and an ambient $[\text{CO}_2]_a$ plot. The soil profiles and agronomic histories were similar between the fields within each block. Rice growth, nutrition, and disease severity of rice blast and sheath blight were evaluated for each plot.

Each of the elevated $[\text{CO}_2]_a$ plots was installed with a FACE ring, which consisted of eight plastic tubes arranged in an octa-

Fig. 1. Free-air enrichment (FACE) apparatus using pure CO_2 injection in the field. Rice plants were exposed in four paddies to elevated CO_2 by growing them within 12-m-diameter rings which sprayed pure CO_2 toward the center from peripheral emission tubes located 50 cm above the canopy. In another four paddies, plants were grown under ambient CO_2 conditions with no ring structures in place.

gonal shape 12 m diameter and suspended 50 to 60 cm above the canopy height (Fig. 1). Pure CO₂ gas was sprayed toward the center from the plastic tubes at the upwind sides; these are referred to as FACE plots. Within the rings, rice plants were grown from transplanting to harvest under elevated [CO₂]_a. The ambient [CO₂]_a plots were located at least 90 m from the nearest FACE ring to minimize CO₂ contamination. Monitoring and control of CO₂ emission was carried out by a series of CO₂ and wind sensors, data loggers, controllers, and valves. The target CO₂ concentration at the center of the FACE plots was 200 µmol mol⁻¹ above that of the ambient plots; enrichment was carried out continuously.

Crop history. Rice (cv. Akitakomachi; susceptible to rice blast) seed were sown in nursery trays (30 by 60 cm) containing fertilizer-impregnated rockwool mats (1.8:1.0:2.0 g/mat; N:P₂O₅:K₂O) at a rate of 120 g/tray. Just after seedling emergence, the trays were placed in plastic chambers fumigated with either ambient air or ambient air plus CO₂ at 200 µmol mol⁻¹ (28). A detailed description of the chambers facility has been provided by Okada et al. (28). The dimensions of the chambers were 6 m (width), 30 m (length), and 3 m (maximum height). The growth room was covered with 0.05-mm-thick ethylene-tetrafluoro ethylene copolymer (ETFE) film (F-CLEAN; Asahi Glass-Tech Co. Ltd., Japan). For control purposes, infrared CO₂ analyzers (ZFP9; Fuji Electric Co., Japan) were used in both the ambient and elevated CO₂ chambers. Seedlings were grown for 14, 23, and 24 days in 1998, 1999, and 2000, respectively. Seedlings at the fifth leaf stage were transplanted in the field plots of corresponding [CO₂]_a on 21 May, 20 May, and 22 May in 1998, 1999, and 2000, respectively. Three seedlings were planted together into a spot (i.e., a "hill"). Hills and rows were 17.5 and 30 cm apart, respectively (equivalent to 19.05 hills m⁻²). In all years, fields were flooded throughout the season, except for a 5-day summer drainage in mid-July and from ≈10 days prior to harvest. N was supplied as ammonium sulfate at three application rates: 4 (low; LN), 8 (medium; MN), and 12 (high; HN) g of N/m² in 1998 and 4, 9, and 15 g of N/m² in 1999 and 2000. Normal N application rate in this region was ≈8 g N/m². To minimize mixing of the paddy water between N treatments, areas receiving LN and HN were separated from the rest of the plot (which received MN) by a 30-cm polyvinylchloride barrier pushed 10 cm into the ground. We only studied the plants in the HN, except for in 2000, when we additionally observed the sheath blight development not only on HN plants but also on LN plants. The total N amount was split applied as a basal fertilizer (63% of the total) and top dressings at mid-tillering (20%) and panicle initiation stages (17%). Phosphate (P) and potash (K) were applied as basal fertilizers at the following rates: P at 13.1 g/m² in 1998 and 2000 and K at 39.8 g/m² in all 3 years. The experimental plants were surrounded by border plants treated similarly to the test plants.

Inoculation and disease assessment of rice blast. An isolate of *M. oryzae*, Naga 69-150, virulent to Akitakomachi, was used for inoculation. For inoculum preparation, the isolate was grown on oat meal agar in petri dishes at 25°C. After mycelial mats had covered the surface of the agar plates, the mats were brushed gently with a toothbrush and then exposed to black light blue fluorescent lamps (FL20S BL-B; Hitachi) for 2 days. The surface then was washed with sterilized distilled water and filtered through two layers of tissue paper to prepare the conidial suspension. The conidial concentration was adjusted to approximately 5 × 10⁵ conidia ml⁻¹ and Tween 20 was added to a final concentration of 0.02% (vol/vol).

Six hills of rice plants in each plot were covered with a transparent polyethylene film with a thickness of 0.07 mm and immediately inoculated with the rice blast fungus in the field just before sunset by spraying 20 ml of the conidial suspension per hill. Polyethylene film was removed just before sunrise of the next day. The film blocked wind and kept moisture inside while

transmitting longwave radiation from the rice plants. Consequently, the rice surface was kept cooler than the surrounding atmosphere, and dew formation on the rice surface was enhanced (21).

Inoculation was done three times a season: at panicle-initiation (PI), panicle-formation (PF), and heading stages. The inoculation at PI was done on 1 July 1998, 8 July 1999, and 3 July 2000, whereas PF was on 22 July 1998, 21 July 1999, and 18 July 2000. The inoculation at heading stage was conducted only in 1999 and 2000 on 9 August for both years. In 1998, a natural occurrence of panicle blast was observed on 15 September in the field without uprooting. Within 3 days after inoculation, before leaf or panicle blast lesions appeared, the rice plants were dug from the ground with an intact root zone and transplanted to plastic pots, which then were moved to a greenhouse under ambient CO₂ condition to prevent the disease from spreading to other plants in the plot. In this project, many experiments (rice growth, yield, plant nutrient, and so on) as well as fungal infection to investigate the effects of elevated [CO₂]_a in the field were carried out in same plot.

Fourteen days after the inoculation, the number of leaf blast lesions was counted for inoculations at PI and PF stages in each plant. The number of healthy and infected spikelets also was counted for each plant inoculated at the heading. Disease incidence was determined by calculating the percentage of diseased spikelets per plant. The values of six plants were used to calculate the average value of each plot.

Disease assessment of sheath blight. Sheath blight developed naturally in all the plots in 1999 and 2000. The disease incidence was assessed in the HN subplots on July 29 1999 and 10 August 2000. Fifty hills per plot were counted in the field, and hills with more than one lesion were regarded as diseased. For the diseased hills, height of the uppermost lesions above the soil surface in leaf sheaths was measured relative to the plant height. In 2000, the same assessment also was made in the LN subplots.

Measurement of leaf N and silicon content. Leaf blades were removed from leaves located at the first and second positions that were counted downward from the top of the plant just before each blast inoculation at PI and PF stages.

The leaf samples were dried at 80°C in a forced-air oven for 1 week. Grounded samples (100 mg each) were subjected to chemical analyses with three replications and two repetitions. Total N was determined by the micro-Kjeldahl method (12). Silicon (Si) contents were determined by a gravimetric method. Samples were burned in platinum crucibles at 500°C and the ash was treated repeatedly with dilute acid (1.5 M HNO₃:3.71 M HCl) to remove other mineral impurities. The silica was filtered out, ignited, and weighed. The weights of silica were converted into those of Si.

Statistical analysis. The observed values for disease severity, incidence, and leaf chemical composition were averaged for each plot and subjected to statistical analysis. The statistical differences between the [CO₂]_a levels were determined with the paired *t* test within each year.

RESULTS

Environmental conditions. Actual season-long average CO₂ concentration in the ambient plots were 368, 369, and 365 µmol mol⁻¹ in 1998, 1999, and 2000, respectively (Table 1). On the other hand, actual season-long average CO₂ concentration in the FACE plots were 635, 650, and 574 µmol mol⁻¹ in 1998, 1999, and 2000, respectively. Although the target CO₂ concentration to be achieved at the center of the FACE rings was 200 µmol mol⁻¹ above that of the ambient level, the concentration of the subplots fluctuated widely because of the changes of both the control algorithm and the subplot allocation in the ring across the years. Temporal [CO₂]_a control was adequate, with 60 and 90% of the air samples at ring center having a [CO₂]_a within 10 and 20% of

the target, respectively. Spatial $[\text{CO}_2]_a$ distribution also was adequate, with 60% of the ring area having a $[\text{CO}_2]_a$ that was within 15% of that the center (29). Air temperature and solar radiation also are summarized in Table 1 for the three growing seasons. Compared with a normal year, temperature was moderate in 1998 but extremely hot in both 1999 and 2000, whereas solar radiation was low in 1998 and high in 1999 and 2000.

Severity of rice blast infection under elevated CO_2 concentration. At PI and PF stages, the severity of leaf blast infection in plants grown in elevated $[\text{CO}_2]_a$ was significantly higher than those in ambient $[\text{CO}_2]_a$ for 1998 and 2000, but not for 1999 (Table 2).

The incidence of panicle blast was significantly higher in plants exposed to elevated $[\text{CO}_2]_a$ than to those exposed to ambient $[\text{CO}_2]_a$ under natural incidence in 1998 (Table 3). By comparison, artificial inoculation at heading stage in 1999 and 2000 caused a significant increase in the incidence of panicle blast, but no differences were detected between plants grown in either elevated or ambient $[\text{CO}_2]_a$ (Table 3).

Incidence and severity of rice sheath blight under elevated CO_2 concentration. Disease incidence and severity of rice sheath blight that occurred naturally were assessed in the field without

uprooting. With the HN application, the incidence of rice sheath blight in plants exposed to elevated $[\text{CO}_2]_a$ was significantly higher compared with ambient $[\text{CO}_2]_a$ for both 1999 and 2000 (Table 4). In the LN subplots, the blight incidence was not significantly different between ambient and elevated $[\text{CO}_2]_a$ in 2000. In addition, the height of the uppermost lesion in the leaf sheath was not significantly different between elevated and ambient $[\text{CO}_2]_a$ in 1999 and 2000, regardless of the N application rate (Table 4).

Leaf Si and N contents under elevated CO_2 concentration. Si content in the rice leaves exposed to elevated $[\text{CO}_2]_a$ was significantly lower compared with ambient $[\text{CO}_2]_a$ in 1998 and 2000 at both PI and PF growth stages (Table 5). The reduction of Si content due to the CO_2 enrichment was 15% (1998) and 16% (2000) at the PI stage, and 13% (1998) and 22% (2000) at the PF stage. In 1999, however, the leaf Si content was not significantly different between ambient and elevated $[\text{CO}_2]_a$. There were no significant differences in leaf N content between the elevated and ambient $[\text{CO}_2]_a$ in any years or growth stages. As suggested by the previous studies (16), the leaf N contents tended to decrease under elevated CO_2 compared with ambient, but it was not significant. This might be due to the fact that the plants were grown at a high-N subplot in these experiments, where a supply of N was ample, especially in early growth stages, or only newly expanded leaves at the top of the canopy were sampled for our analysis. For similar leaves in term of growth stage and leaf age, Seneweera et al. (35) reported that the decrease in leaf N content under elevated CO_2 was significant at a low and medium N level, but insignificant at a high N.

TABLE 1. Summary of CO_2 concentration, mean daily temperature, and solar radiation in the field during the growing season^a

Conditions	Year			
	1998	1999	2000	Normal ^b
Ambient CO_2 ($\mu\text{mol mol}^{-1}$)	368	369	365	...
Elevated CO_2 ($\mu\text{mol mol}^{-1}$)	635	650	574	...
Temperature ($^{\circ}\text{C}$)	19.7	21.1	21.4	19.3
Solar radiation ($\text{MJ D}^{-1} \text{m}^{-2}$)	12.5	15.3	14.8	14.8

^a Mean values are calculated for a period from transplanting to maturity.

^b Because there are no statistics observed for long years at the free-air CO_2 enrichment site, the values for a normal year are collected at the closest weather stations: temperature in Shizuikuishi and solar radiation in Morioka, 7 and 20 km from the site, respectively.

TABLE 2. Number of leaf blast lesions per plant grown in ambient and elevated CO_2 conditions

Year	Inoculation growth stage of rice plants ^a			
	Panicle initiation		Panicle formation	
	Ambient	Elevated	Ambient	Elevated
1998	86.88	142.88*	24.25	33.75*
1999	26.33	26.65	5.87	6.14
2000	17.81	24.94*	7.06	9.31*

^a Leaf blast inoculations were made at panicle initiation on 1 July 1998, 8 July 1999, and 3 July 2000 and at panicle formation on 22 July 1998, 21 July 1999, and 18 July 2000. Statistical differences between the $[\text{CO}_2]_a$ levels were determined with the paired t test for each year. Means followed by * are significant at $P < 0.05$.

TABLE 3. Panicle blast incidence (%) under ambient and elevated CO_2 conditions

Year	Ambient	Elevated ^a
1998 ^b	1.1	2.6*
1999 ^c	23.9	20.3
2000	47.4	46.3

^a Statistical differences between the $[\text{CO}_2]_a$ levels were determined with the paired t test within each year. Means followed by * are significant at $P < 0.05$ between ambient and elevated conditions.

^b Panicle blast incidence occurred naturally and was determined by estimating the disease severity through field observations on 15 September 1998.

^c Rice plants were artificially inoculated on 9 August 1999 and 2000. The number of healthy and infected spikelets was counted for each plant. Disease incidence was determined by calculating the percentage of diseased spikelets.

TABLE 4. Sheath blight incidence and severity under ambient and elevated CO_2 concentrations in the field^a

Year ^b	N ^c	Diseased plants (%) ^d		Lesion height (%) ^e	
		Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
1999	High	3.2	10.1*	21.5	24.5
2000	High	20.1	40.3*	41.2	41.2
2000	Low	13.4	10.3	33.3	36.4

^a Statistical differences between the $[\text{CO}_2]_a$ levels were determined with the paired t test within each year. Means followed by * are significant at $P < 0.05$ between ambient and elevated conditions within each year and nitrogen (N) rate.

^b Disease incidence and severity was assessed on 29 July 1999 and 10 August 2000 in the field.

^c N was applied as ammonium sulfate at rates of 15 and 4 g/m² for high- and low-N subplots, respectively. Normal rate of N application was 8 g/m².

^d Percentage of hills with more than one lesion.

^e Height of the uppermost lesion above the soil surface relative to the plant height.

TABLE 5. Leaf silicon (Si) and total nitrogen (N) contents under ambient and elevated CO_2 concentrations^a

Year	GS ^b	Si (% by dry weight)		N (% by dry weight)	
		Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
1998	PI	2.23	1.89*	4.14	4.24
	PF	2.28	1.98*	2.85	2.63
1999	PI	2.00	2.10	4.47	4.27
	PF	2.03	1.90	3.87	3.41
2000	PI	1.93	1.62*	3.99	3.7
	PF	2.23	1.74*	2.78	2.85

^a Statistical differences between the $[\text{CO}_2]_a$ levels were determined with the paired t test within each growth stage and year. An asterisk (*) indicates that the means are significantly different at $P < 0.05$ between the ambient and elevated CO_2 concentrations.

^b GS = growth stage. Leaf samples were collected just before leaf blast was inoculated to plants at two GSs in the field, panicle initiation (PI) and panicle formation (PF). Leaf blades were removed from leaves located at the first and second positions that were counted downward from the top of the plant and subjected to the Kjeldahl (N) and gravimetric (SiO_2) methods.

DISCUSSION

Research over the past few years suggests that the most likely impact of elevated $[\text{CO}_2]_a$ on plant disease epidemics would be mediated through changes in the host physiology and morphology (1). With leaf blast, we found that the plants grown in elevated $[\text{CO}_2]_a$ were more severely infected than plants grown in ambient $[\text{CO}_2]_a$. However, no significant difference was detected between the $[\text{CO}_2]_a$ in 1999. Because elevated $[\text{CO}_2]_a$ is unlikely to inhibit directly the penetration of *M. oryzae* into the leaf surface, the above result would be mediated through changes in physiology of rice plants to leaf blast under elevated $[\text{CO}_2]_a$. The severity of leaf blast infection was considerably higher in 1998 than in 1999 and 2000 regardless of $[\text{CO}_2]_a$. These differences in the severity may be attributed to more favorable weather condition for infection after artificial inoculation observed in 1998 compared with 1999 and 2000. With rice panicle blast, on the other hand, the effect of elevated $[\text{CO}_2]_a$ on infection was unclear. In 1999 and 2000, no significant differences were observed for disease incidence between ambient and elevated $[\text{CO}_2]_a$. In 1998, the difference was statistically significant, but the overall disease severity was very low. To our knowledge, this research is the first to show the effect of elevated $[\text{CO}_2]_a$ on rice diseases, and the first to show that the elevated $[\text{CO}_2]_a$ could enhance disease occurrence.

The mechanisms by which elevated $[\text{CO}_2]_a$ may increase the susceptibility of rice plants to leaf blast have not been examined. However, N and Si nutrition can greatly affect rice susceptibility to blast disease (3,10,11,30) and, hence, could be altered as a result of increased $[\text{CO}_2]_a$. Various studies have shown that elevated $[\text{CO}_2]_a$ lowers stomatal conductance of plants and reduces transpiration rate (12,31,34,41). Although uptake of Si by rice roots is an active process associated with energy consumption (25), transport of Si from roots to leaves is believed to be by transpiration, a passive system. The decline in leaf transpiration rate in elevated $[\text{CO}_2]_a$ might lower Si transport to leaves and, hence, the Si accumulation at the leaf surface. The reduction of Si content in the leaves would predispose the rice plants to infection by *M. oryzae*, because Si often is associated with blast resistance. On the other hand, Hibberd et al. (4–6) found that the primary penetration of *Erysiphe graminis* in barley was reduced under $[\text{CO}_2]_a$ at $700 \mu\text{mol mol}^{-1}$. They attributed the result to higher rates of net photosynthesis that increased in defense-related compounds mobilized into the resistance reaction that included the production of papillae and accumulation of Si at the sites of penetrating appressoria.

A few mechanisms have been proposed for Si-induced resistance to *M. oryzae* in rice. One is the so-called mechanical barrier hypothesis. Si is accumulated just beneath the cuticle of epidermal cells of the rice leaf surface (16,43,44), and this layer is believed to physically prevent the *M. oryzae* from penetrating through the epidermal cell walls (11,17,36,43). Another hypothesis emphasizes resistance reactions induced by Si (32). It is possible that both mechanisms could work simultaneously.

In our FACE experiments, the transpiration rate and stomatal conductance of the rice plants were lower in elevated $[\text{CO}_2]_a$ (16), and, in 1998 and 2000, leaf Si contents were significantly lower in the elevated $[\text{CO}_2]_a$ treatments than in ambient $[\text{CO}_2]_a$. In 1999, on the other hand, no significant difference was found in Si content between elevated and ambient $[\text{CO}_2]_a$ treatments. Thus, the change in leaf Si content due to the elevated $[\text{CO}_2]_a$ shows a close association with blast susceptibility, whereas a causal relationship between these changes has yet to be shown. Additional research is required to establish the direct link between lower Si content and higher blast susceptibility under elevated $[\text{CO}_2]_a$. Such research would need to show that (i) the cuticle-silica double layer of rice leaves is thinner or sparser in leaves grown under elevated $[\text{CO}_2]_a$ than those grown under ambient $[\text{CO}_2]_a$ (40) and (ii) with inoculation experiments, conidial penetration by

M. oryzae into the epidermal cells of rice leaves is more frequent in plants grown under elevated $[\text{CO}_2]_a$ than those grown under ambient $[\text{CO}_2]_a$. These hypotheses currently are being tested with the FACE system.

It remains unclear why there was no significant effect of elevated $[\text{CO}_2]_a$ in 1999. The weather condition of the FACE site during June to July 1999 was characterized by a longer light duration and lower humidity than in average years. Although not tested, these weather conditions might have accelerated Si transport from the root to the upper part of the rice plant, raising the Si content in rice leaves to its maximum level.

Several studies have shown that excessive application of N fertilizer increases the susceptibility of rice to blast development (10,22–24,30). Thompson et al. (37) found that a lower N content in wheat plants under elevation of $[\text{CO}_2]_a$ was associated with lower disease severity of powdery mildew. Thus, we had anticipated that elevated $[\text{CO}_2]_a$ would make rice plants more resistant against the blast disease, because elevated $[\text{CO}_2]_a$ tends to lower N concentration in crop plants (19). In our studies, however, there was no significant effect of $[\text{CO}_2]_a$ on leaf N content; accordingly, N nutrition likely does not explain the change in rice susceptibility to blast under elevated $[\text{CO}_2]_a$. Host water content also may influence the severity of leaf blast infection. Host water deficits reduced the sporulation of fungus (42). Under drought conditions, elevated $[\text{CO}_2]_a$ did not change the N content but increased water content in wheat plants and the disease severities of powdery mildew (37). Similarly, with a C_4 grass, *Spartina patens*, elevation of $[\text{CO}_2]_a$ increased water content in the plants and increased the disease severity of fungal infection without a change in N content (38). Future work will be needed to determine the water content of the plants grown in ambient and elevated $[\text{CO}_2]_a$.

Manning and Tiedemann (26) predicted that elevated $[\text{CO}_2]_a$ would lead to greater dry matter and denser canopy, and that these changes would make the host plants more conducive to the progression of various diseases as a change in host morphology. In fact, $[\text{CO}_2]_a$ at $700 \mu\text{mol mol}^{-1}$ conferred a degree of tolerance of tomato plants to infection by *Phytophthora parasitica* (13). They suggested that increased vegetative growth in both the leaf and root systems under higher $[\text{CO}_2]_a$ might have compensated for the loss of growth caused by the root pathogen. Disease incidence of sheath blight was significantly higher in elevated $[\text{CO}_2]_a$ than in ambient $[\text{CO}_2]_a$ when a high level of N was applied. These differences in disease incidence may be attributed to the difference of tiller density within the rice canopy. Several experiments using controlled environmental facilities have demonstrated that rice plants develop more tillers in elevated $[\text{CO}_2]_a$ than in ambient $[\text{CO}_2]_a$ (8,14,46). At the high-N subplots in our FACE experiments, rice plants exposed to elevated $[\text{CO}_2]_a$ developed significantly more tillers per hill than those in ambient $[\text{CO}_2]_a$ (15,16). As the number of tillers per hill increased, the tiller density per area increased. Therefore, sclerotia of the pathogen floating on the water surface would cause more frequent infections if more tillers were present. Accordingly, rice plants exposed to elevated $[\text{CO}_2]_a$ should have higher initial incidence of sheath blight. In addition, elevated $[\text{CO}_2]_a$ may enhance horizontal spread of the disease from diseased sheaths to the surrounding tillers. Because the disease spreads across the tillers via runner hyphae, denser tillers should make the disease spread more readily. In contrast, under low N, development of the tillers in elevated $[\text{CO}_2]_a$ was similar to that in ambient $[\text{CO}_2]_a$ (15,16), and there was no significant difference in sheath blight incidence between elevated and ambient $[\text{CO}_2]_a$ in the 2000 season. While this comparison was done only for 2000, it is possible that elevated $[\text{CO}_2]_a$ does not alter directly the incidence of sheath blight, but that higher tiller density due to elevated $[\text{CO}_2]_a$ is the primary cause of the higher disease incidence. We did not record data on the initial disease incidence or the temporal disease

progression on the incidence; therefore, the above explanation remains a hypothesis. Further observations on sheath blight disease progression are necessary to determine the validity of this hypothesis.

Although a significant effect of $[\text{CO}_2]_a$ on the horizontal spreading of sheath blight was observed, upward spread of the sheath blight lesions was not influenced by $[\text{CO}_2]_a$. This result is not consistent with earlier reports. For example, Rodrigues et al. (33) showed that application of Si as a fertilizer to rice plants increased the Si content in the straw with a significant reduction in sheath blight severity, which is represented as the relative lesion height of the whole plant. In our study, Si contents in leaf blades grown in elevated $[\text{CO}_2]_a$ were lower than that in ambient $[\text{CO}_2]_a$. However, there was no change in the level of disease in terms of maximum lesion height. The decrease in leaf Si content in elevated $[\text{CO}_2]_a$ in this FACE experiment may not have been large enough to change susceptibility of the plants to sheath blight, or the elevated $[\text{CO}_2]_a$ may not have changed Si content in leaf sheaths, which was not measured in this experiments.

In summary, this study showed that elevated $[\text{CO}_2]_a$ increased leaf blast and sheath blight development. Hence, the potential risks of rice blast and sheath blight would increase in rice grown under elevated $[\text{CO}_2]_a$. The decline of Si in rice plants due to elevated $[\text{CO}_2]_a$ may enhance susceptibility to blast, and the change of rice canopy structure may accelerate spread of sheath blight in the field. Although crop growth and yield tend to respond positively to elevated $[\text{CO}_2]_a$ (19), the increased risks of the plant to diseases need to be considered in predicting the impacts of global increase in atmospheric $[\text{CO}_2]_a$ on crop production in the future.

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