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SHORT COMMUNICATION / *COMUNICAÇÃO*

Elevated atmospheric CO_2 concentration increases rice blast severity

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ABSTRACT

The predicted atmospheric carbon dioxide concentration's increases are likely to alter plant-pathogen interactions. To assess the effects on rice blast, during three years (2007, 2008 and 2009), three rice cultivars (Agulha Precoce, Shao Tiao Tsao and Caloro) were exposed to elevated CO_2 air concentration (approximately 100 - 300 µmol mol^{-1} higher than ambient) in open-top chamber facility. The disease was more severe under high CO_2 concentration. Area under disease progress curve was 35.43 under high CO_2 concentration and 17.48 for the current concentration. Leaves of treated plants tended to contain less silicon. In 2009, plant height of two cultivars was greater in high CO_2. Understanding and predicting the climate-driven changes in the agroecosystem may allow the development of adaptation strategies in order to minimize crop losses.

Key words: *Magnaporthe oryzae*, *Pyricularia oryzae*, *Oryza sativa*, carbon dioxide, climate change.

The importance of the environment for the occurrence of plant diseases has been known for over two thousand years (Colhoun, 1973). Climatic variables such as temperature, relative humidity, solar radiation, soil moisture, and others can influence plant growth and resistance as well as plant-pathogen interactions, pathogen reproduction, dispersal and survival. Among climate change projections, IPCC (2007) indicated that atmospheric CO_2 concentration will increase over this century, reaching 730-1020 µmol mol^{-1} by 2100. Annual emissions increased 80% from 1970 to 2004, exceeding the natural scale of the last 800,000 years (IPCC, 2007; Lüthi et al., 2008).

Predicted impacts of elevated CO_2 atmospheric concentration on rice (*Oryza sativa* L.) raise serious questions concerning food security for the future (Horie et al., 2000; Eastburn et al., 2011). Rice blast, caused by *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*) (T.T. Hebert) M.E. Barr, is the most important disease of upland rice, from the seedling up to harvest growth stage. Under favorable conditions, the disease causes the death of the leaves and often the whole plant. Despite the importance of the rice crop and the predicted rise in atmospheric CO_2, few studies have evaluated the effects of increased CO_2 concentration on rice blast. The pathosystem was earlier studied in a Free Air CO_2 Enrichment (FACE) facility in northern Japan by Kobayashi et al. (2006). In that study, leaf blast lesions of a susceptible rice cultivar (cv. Akitakomachi) were higher at elevated CO_2 concentrations (200-280 µmol mol^{-1} above ambient) in two of the three years of the study. Lower leaf silicon content may have contributed to the increased susceptibility to leaf blast under elevated CO_2 concentration. The authors discussed that the decline in leaf transpiration rate under elevated CO_2 concentration might lower Si transport to leaves and, hence, the Si accumulation at the leaf surface. The close relationship between Si and blast susceptibility (Sun et al., 2010) suggests that reduction of Si content in leaves would predispose rice plants to infection by *M. oryzae*. By contrast, elevated CO_2 levels had little or no effect on incidence levels of the panicle blast phase of the disease.

Despite the general evidence of beneficial effects of CO_2 on the host plant (Pritchard & Amthor, 2005), it is not known if these effects will persist in the presence of pathogens or other limiting factors, particularly in tropical countries (Ghini et al., 2011). According to Chakraborty & Newton (2011), little empirical research on plant diseases under field conditions has realistically mimicked climate change, and this lack severely hampers the development of crop adaptation alternatives or options for disease management under climate change.

The objective of this study was to evaluate the effect of atmosphere CO_2 level increase on plant growth and blast disease severity.

The field experimental facility (open-top chambers - OTC) was built in Jaguariúna, state of São Paulo, Brazil (latitude 22°14'10"S, longitude 46°59'09"W, altitude 570 m a.s.l.). The OTCs (diameter 1.9 m, height 2 m) were constructed from a steel frame, covered with transparent polyethylene film with a UV-light stabilizer additive. The OTCs were equipped with a frustum at the top (diameter 1.25 m, height 33 cm) to deflect air and prevent dilution of the desired CO_2 concentration (Lessin & Ghini, 2009). CO_2

concentrations were controlled by an infrared gas analyzer (IRGA, PP Systems, model WMA-4), which sampled air from the plots at 10-min intervals, through a pipe placed 50 cm above ground. Air temperature was recorded and stored in a data logger. The plots were irrigated using a sprinkler system during drought periods.

The treatments were: OTCs with enriched CO_2 atmosphere (OTC+CO_2), OTCs with ambient CO_2 concentration (OTC-A), and unenclosed control (C). A randomized block design was used with three replicates. Each OTC was an experimental unit. Mean diurnal CO_2 concentrations were 451, 477, and 544 µmol mol^{-1} respectively in the C, OTC-A, and OTC+CO_2 over the three years (Table 1). Treatments with OTC showed higher diurnal temperatures than the control treatment (C). However, temperatures of OTCs with and without gas injection were similar.

In 2007, the Agulha Precoce rice cultivar was sown in total area of the plots. In 2008, Agulha Precoce, Caloro and Shao Tiao Tsao cultivars were sown in rows. In 2009, Agulha Precoce and Shao Tiao Tsao cultivars were again sown in rows. The plots were divided in one half and two quarters. In each division 5 rows were planted, 15 cm apart, being Agulha Precoce in the half, and Shao Tiao Tsao and Caloro in each of the quarters. Rice seeds were sown in the plots on December 19th 2006, December 28th 2007 and January 5th 2009.

In 2007, the soil was fertilized with 300 kg ha^{-1} of NPK (10-10-10) and 25 kg ha^{-1} of ammonium sulfate. In 2008, 7 L m^{-2} of cattle manure was applied with mineral fertilizer of the same composition applied in 2007. In 2009, the soil was not fertilized because nutrient levels were adequate. After emergence, plants were fertilized weekly with urea, at 10 g m^{-2}.

Plant height was measured 23, 32 and 53 days after emergence (DAE) in 2008 and 32, 45 and 60 DAE in 2009. Plant dry mass was determined at 32 and 79 DAE in 2008; and at 32, 45 and 60 DAE in 2009. The mass of panicles and grain yield was determined at 88 and 86 DAE in 2008 and 2009, respectively. The colorimetric analysis described by Korndörfer et al. (1999) was used to analyze the Si content of leaves.

Inocula of *M. oryzae* (strains P01, 2449 and 1365) were produced by growing the isolates on oatmeal agar (15 g rice bran, 15 g oatmeal, 5 g dextrose, 20 g agar and distilled water q.s.p. 1000 mL) in Petri dishes at 27°C for 11 days. After mycelial growth covered the agar surface, each plate received 3 mL of sterile distilled water, and the aerial mycelia were gently brushed with a Drigalski spreading spatula. Then, the fungus was exposed to daylight fluorescent lamps for two days at 27°C. The surface was washed with 10 mL of sterilized distilled water with Tween 20 (0.02% v/v), and the resulting suspension was filtered through two layers of cheesecloth to prepare the conidial suspension. The conidial concentration was adjusted to approximately 6 x 10^5 conidia mL^{-1}.

In 2007, the plants were not inoculated because blast occurred naturally. In 2008, although blast again occurred naturally, all plants were inoculated at 30, 35 and 45 DAE, with 40, 50 and 60 mL of conidial suspension per plant, respectively. In 2009, all plants were inoculated with *M. oryzae* at 5, 10, 25, 32 and 39 DAE, just before sunset, by spraying 2, 5, 20, 35 and 40 mL of conidial suspension per plant, respectively.

Blast severity, expressed as percentage of diseased leaf area, was recorded for all completely unfolded leaves, the main tillers for ten plants of the three central rows per plot, at 32, 41, 43, 45, 48 and 52 DAE in 2007; 29, 32, 35, 39, 44, 53 and 59 DAE in 2008; and 32, 38, 45, 53 and 60 DAE in 2009. A diagrammatic scale (0, 0.5, 1, 2, 4, 8, 16, 32, 64 and 82% of diseased leaf area) was used to assess disease severity (Notteghem, 1981). The area under the disease progress curve (AUDPC) was calculated.

In 2008, the percentage of diseased plants per plot was obtained by counting the total number of plants and the number of plants with symptoms in the tissue of the nodal region and dead parts above this area, characterizing the presence of *M. oryzae* in plant tillers in the Shao Tiao Tsao cultivar, 111, 118 and 126 DAE. In 2008 and 2009, the incidence of *M. oryzae* and other fungi on Shao Tiao

TABLE 1 - Summary of CO_2 concentration, and mean day and night temperatures in the treatments during three growing seasons

	[CO_2] (µmol mol^{-1})				**Temperature (°C)**		
Years	**C^1**	**OTC-A**	**OTC+CO_2**		**C**	**OTC-A**	**OTC+CO_2**
				Day			
2007	438	460	559		25.8	28.8	28.1
2008	444	473	510		25.3	28.7	27.8
2009	473	500	564		26.4	29.8	30.3
				Night			
2007	470	517	733		21.9	22.5	22.4
2008	489	532	633		20.1	20.5	21.4
2009	534	574	727		21.3	22.1	22.0

^{1}C, control; OTC-A, open-top chambers with ambient CO_2; OTC+CO_2, OTC with elevated CO_2.

Tsao seeds was evaluated by blotter test with deep freezing (Neergaard, 1977).

Statistical analyses were performed using the Sisvar program. To compare the effect of cultivars, CO_2 treatments and their interaction in 2008, data were submitted to analysis of variance ($p \leq 0.05$). Means were compared by Tukey test ($p \leq 0.05$).

The increase in CO_2 concentration had no effect on the height of the three rice cultivars in 2008 (Table 2). However, in 2009, the plant height of cultivars Agulha Precoce and Shao Tiao Tsao was higher in enriched CO_2 atmosphere. For Shao Tiao Tsao plants, panicles emerged and the crop matured 2-3 days earlier under elevated CO_2 atmosphere, and 4-5 days earlier than Agulha Precoce plants in 2008 and 2009 (data not shown). CO_2 enrichment did not increase plant dry mass of Agulha Precoce and Shao Tiao Tsao cultivars, although higher values of plant dry mass were always observed in the treatment with elevated CO_2 (Table 2). The increase in CO_2 concentration did not affect the seed weight per panicle or the weight of 100 seeds (data not show). In 2007 and 2009, Agulha Precoce plants did not produce seeds because panicles were severely attacked by blast fungus; and the same occurred in 2008, for Caloro. In 2009, the leaves of Agulha Precoce plants grown in elevated CO_2 trended to accumulate less Si than those submitted under ambient condition. Si contents were 4.4%, 4.7% and 3.5%, respectively, in the C, OTC-A and OTC+CO_2 treatments. In 2008, however, this trend was not observed (Si contents were 2.5%, 2.1% and 2.1%, respectively).

High CO_2 air concentration increased the severity of the disease when the three cultivars were analyzed jointly within three years of evaluation (Table 3). Agulha Precoce plants showed the highest AUDPC in the OTC+CO_2 treatment in 2007 and 2009, but not in 2008. Caloro plants showed an increase in AUDPC with elevated CO_2 concentration in 2008. There was no statistically significant ($p \leq 0.05$) interaction among cultivars and CO_2 treatments (F value = 1.15). In the same year, blast at the nodes occurred in the cultivar Shao Tiao Tsao, and the AUDPC were 163.5, 791.4 and 667.4 for treatments C, OTC-A and OTC+CO_2, respectively. High CO_2 concentration did not alter the occurrence of disease in the nodal region, since OTC+CO_2 and OTC-A did not differ statistically from the control.

High CO_2 concentration did not alter the occurrence of *M. oryzae*, *Bipolaris oryzae* (Breda de Haan) Shoemaker, *Phoma sorghina* (Sacc.) Boerema, Derenbosh & Van Kesteremn, *Drechslera* spp., *Alternaria* spp. or *Microdochium oryzae* (Hashioka &Yokogi) Samuels & I.C. Hallett in seeds of Shao Tiao Tsao in 2008 and 2009 (data not shown).

Elevated CO_2 concentration increased blast severity in three rice cultivars for all three years of the study (Table 3). Similar results were obtained by Kobayashi et al. (2006), using a FACE facility in Japan. These authors suggested that the increased severity of disease was a result of reduced Si concentration in plants grown in a CO_2 -enriched environment, due to the reduction of respiration rates. Likewise, in our study in 2009, the Si concentration decreased in leaves of plants grown with CO_2 enrichment (OTC+CO_2). Silicon concentration is a factor in the susceptibility of rice plants to *M. oryzae*, as suggested by Datnoff et al. (1997). In 2008, there was no difference in the Si content and disease severity in the leaves of the cultivar Agulha Precoce, as observed in 2009, probably because of lower concentrations of CO_2 in treatment OTC+CO_2 in 2008 (Table 1).

The mechanism by which rice roots remove Si from the soil is an active process associated with energy consumption, but Si transport from roots to leaves is a passive process governed by the transpiration rate. The

TABLE 2 - Effect of enriched CO_2 concentration on plant height (H) and plant dry mass (PDM) of the three rice cultivars (Agulha Precoce, Shao Tiao Tsao and Caloro), in 2008 and 2009

Treatments	**Agulha Precoce**		**Shao Tiao Tsao**		**Caloro**	
	H[2] (cm)	**PDM (g)**	**H (cm)**	**PDM (g)**	**H (cm)**	**PDM (g)**
			2008			
C[1]	69.1b[3]	6.6b	68.9b	7.4b	52.9b	7.8a
OTC-A	88.6a	10.3a	86.5a	10.4ab	62.9a	7.6a
OTC+CO_2	91.5a	10.6a	81.3a	12.8a	63.1a	6.1a
CV (%)	10.2	9.9	10.2	18.4	10.9	31.8
			2009			
C	69.9c	4.6a	71.1c	5.8a	-	-
OTC-A	91.8b	6.5a	85.7b	7.9a	-	-
OTC+CO_2	104.2a	7.7a	101.2a	11.2a	-	-
CV (%)	9.4	31.8	10.5	33.3	-	-

[1]C, control; OTC-A, open-top chambers with ambient CO_2; OTC+CO_2, OTC with elevated CO_2; [2]Evaluations conducted 53 and 61 DAE (days after emergence) for H and 79 and 60 DAE for PDM for the years 2008 and 2009, respectively. [3]Means followed by the same letter in columns do not differ significantly (Tukey $p \leq 0.05$).

TABLE 3 - Effect of enriched CO_2 concentration on rice blast severity expressed by area under disease progress curve (AUDPC) in leaves of three rice cultivars (Agulha Precoce, Shao Tiao Tsao and Caloro)

Years	Cultivars	AUDPC		
		C[1]	OTC-A	OTC+CO_2
2007	Agulha Precoce	11.96c[2]	35.45b	71.13a
2008	Agulha Precoce	13.15a	15.67a	16.08a
	Shao Tiao Tsao	19.96a	27.71a	24.00a
	Caloro	25.62b	11.40b	72.15a
2009	Agulha Precoce	9.31b	9.60b	22.04a
	Shao Tiao Tsao	6.11ab	5.09b	7.17a
Means		14.35b	17.48b	35.43a

*C, control; OTC-A, open-top chambers with ambient CO_2; OTC+CO_2, OTC with elevated CO_2. [2]Means followed by the same letter in rows do not differ significantly (Tukey $p \leq 0.05$).

decline in transpiration rates at elevated CO_2 may decrease Si transport into the leaves and thus reduce Si accumulation in the plant. Reduced Si content in the leaves may predispose rice plants to infection by *M. oryzae*, because Si is usually associated with resistance to rice blast (Buck et al., 2008).

Chakraborty et al. (2008) argued that recent studies suggest that most of the impacts of rising atmospheric CO_2 on plant diseases may occur due to changes in the anatomy, morphology, phenology and physiology of the plant host. Thus, there is a need for studies with CO_2 enrichment that investigate these phenomena (Ghini et al., 2012). For many plants, increases in photosynthesis and biomass production at elevated CO_2 concentrations have been reported (Pritchard & Amthor, 2005). Ainsworth (2008) conducted a meta-analysis that synthesized research on rice responses to rising atmospheric CO_2 concentration. The results of this analysis showed that an elevated concentration of CO_2 (627 µmol mol^{-1}) increased rice yields by 23%, although diseases were excluded in the studies analyzed. Our results showed that plants grown in OTCs (OTC+CO_2 and OTC-A treatments) showed greater growth (Table 2). However, despite an upward trend in the growth characteristics evaluated in the treatment OTC+CO_2, only plant height, in the experiment conducted in 2009, showed statistically significant differences.

The effect of elevated CO_2 concentration on plant growth may also have been affected by blast severity (Tables 2 and 3). The AUDPC of plants from the OTC+CO_2 treatment was generally higher than from the OTC-A treatment. The higher severity of the disease under elevated CO_2 may have reduced the stimulus in plant growth resulting from CO_2 enrichment.

The combined effect of CO_2, temperature and other environmental variables, such as the level of available nutrients in the soil, can affect the plant response to increased CO_2 concentration. Cheng et al. (2009) conducted a pot experiment in four controlled-environment chambers with two levels of CO_2 (ambient, 380 µmol mol^{-1}; and elevated, 680 µmol mol^{-1}) and two levels of night temperature (22 and 32°C). The authors observed that the whole plant and stem dry weight were increased by both elevated CO_2 and high night temperature, while the ear dry weight was increased by elevated CO_2 and decreased by high night temperature. The results indicated that high night temperature will reduce the stimulatory effect of elevated CO_2 on rice production in the future, if both factors continue to increase. The night temperature observed in this study remained between 20.0 and 22.5°C, and therefore these temperatures did not possibly influenced the results obtained.

Several studies have shown that increased temperature may reduce the mass of grains even under high levels of CO_2 (Horie et al., 2000), while increasing spikelet sterility (Jagadish et al., 2007). Baker (2004), studying the effect of elevated CO_2 (700 µmol mol^{-1}) under different temperature regimes (24, 28, 32, 36 and 40°C), found no increase in grains with enriched CO_2 atmosphere. Moreover, a two-year study conducted in a FACE showed that elevating the concentration of atmospheric CO_2 increased rice productivity (Kim et al., 2003; Yang et al., 2006; Shimono et al., 2008). The authors also reported that the rate of productivity increase varies in different cultivars, ranging from none to a 23% increase in productivity. However, in our study, CO_2 enrichment produced no differences in productivity (Table 2).

Our study indicated that high CO_2 concentration induced changes in rice plants that likely contributed to the observed changes in disease expression. Since the changes differed among cultivars, there is a need to study the behavior of each variety and to introduce this evaluation in the development of new rice cultivars. Understanding and predicting the climate-driven changes in the agroecosystem may allow the development of adaptation strategies in order to minimize crop losses (Ghini et al., 2012).

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