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Effect of elevated atmospheric CO_2 and temperature on the chemical and biological control of powdery mildew of zucchini and the Phoma leaf spot of leaf beet

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Abstract The effects of increased temperature and CO_2 levels on the effectiveness of four fungicides and one microbial treatment on disease development in zucchini-*Podosphaera xanthii* and leaf beet-*Phoma betae* pathosystems, kept under phytotron conditions, have been evaluated in this study. Six CO_2 and temperature combinations have been tested for each pathosystem in four experimental trials.Penconazole and sulphur treatments, applied under a simulated CO_2 and temperature increase scenario, have shown an efficacy in powdery mildew control that ranged from 85.0 to 88.9 % for penconazole and from 89.9 to 92.6 % for sulphur, and the treatments have therefore resulted to be equally effective compared to that observed under 400-450 ppm conditions. The disease control provided by *A. quisqualis* was significantly improved under an increased CO_2 concentration of 800-850 ppm at 26-30°C, by 23.3% for disease incidence and 22.8 % for disease severity. The effectiveness of both mancozeb and azoxystrobin against Phoma leaf spot is affected by high levels of CO_2. The efficacy of mancozeb and azoxystrobin has been improved significantly by 15.3% and 20.6%, respectively, under 800-850 ppm of CO_2 and 22-26°C, compared to the efficacy observed under standard conditions of CO_2. More attention should be paid to the efficacy of chemical and biological control measures considering the predicted future climate changes.

Key words *Ampelomyces quisqualis*, fungicides, climate change, *Podosphaera xanthii*, *Phoma betae*

Although there is ample evidence that the global climate is changing, there is still no consensus on the nature, magnitude, long-term impacts and geographical distribution of such changes (Michaels and Balling, 2009). Carbon dioxide and temperature are both key variables that affect plant and disease development, and different approaches have been used to study the effect of climate changes on plant diseases. It has been estimated that the atmospheric CO_2 concentration at the end of this century will increase from the current level of about 380 ppm to between 500 and 800 ppm, depending on the future emissions scenarios (Meehl et al., 2005), also affecting plant diseases (Chakraborty 2013; Gautam et al. 2013). Over the last decade, the effects of increased temperature and CO_2 on plant diseases have been studied, under phytotron conditions, in several pathosystems (Ainsworth and Long 2005; Chakraborty 2005; Garrett et al. 2006; Grünzweig 2011; Pugliese et al. 2012a,b; Ferrocino et al. 2013; Singh et al. 2014; Gilardi et al. 2016; Chitarra et al. 2015).

An analysis of the impacts of climate changes on plant systems should consider the potential interactions of several different factors, including disease management strategies. Although the use of chemicals for disease control is common practice, their effectiveness may be affected by climate changes, due to the influence on pathogen epidemiology, host susceptibility and the behavior of the chemicals on and in the plant (Coakley et al. 1999; Ghini et al. 2008; Juroszek and von Tiedemann 2011).

Phoma leaf spot and powdery mildew are typical key foliar diseases of various economically important crops in Mediterranean countries, where they cause severe losses in open fields and in greenhouses.

Phoma betae [*Pleospora betae*] (de Gruytera et al. 2009) is a seed-borne, sugar beet (*Beta vulgaris* var. *saccharifera* L.) pathogen, which has recently been observed in Italy on leaf beet (*Beta vulgaris subsp. vulgaris* L.) (Garibaldi et al. 2007), an economically important leafy vegetable commercialized as fresh-cut leaves. No cultivar is known to be resistant or tolerant to *P. betae* (Gilardi et al., 2009), and chemical control is the only known effective strategy on cultivations grown under field conditions. Azoxystrobin, which belongs to the Quinone outside inhibitor (QoI) fungicide family, has been applied extensively to leafy vegetables in Italy, while the use of mancozeb is prohibited on leaf beet, although its use has been expanded to other herbs (minor crops) such as rocket, basil and parsley (Gullino et al. 2010).

Powdery mildew, which is incited by *Podosphaera xanthii* (previously known as *Sphaerotheca fuliginea* and *S. fusca*), is a severe cucurbit disease throughout the world (Sitterly 1978), commonly managed by using resistant cultivars and fungicides. Biological control agents and natural compounds have been proposed and evaluated as possible alternatives to the use of chemicals in numerous pathosystems, and have shown different and mostly limited degrees of success. Among the various biocontrol agents suggested for use against powdery mildews, *A. quisqualis* has been accepted for use in several countries (Jarvis and Slingsby 1977; Copping 2004; Gilardi et al. 2008).

Fungicides and biological control agents may be affected by climate changes. The impact of combined environmental factors, such as temperature and CO_2, on the most commonly adopted strategies to control powdery mildew of zucchini and Phoma leaf spot of leaf beet is basically unknown. The present study has used phytotrons, in which the temperature and CO_2 concentration have been manipulated, in order to evaluate the effect of possible future climate change scenarios on the efficacy of chemical and biological control measures against the powdery mildew of zucchini, and the leaf spot of leaf beet. Both diseases require the adoption of solid management strategies, because severe epidemics and crop losses could occur without effective control measures.

Four experimental trials have been carried out, for each pathosystem, as replicated studies in phytotrons under completely controlled environmental conditions (Table 1). During the experiments, each phytotron was maintained at a relative humidity, RH, of 85-95 %. A 14/10-h day/night photoperiod was provided by means of two lighting systems (master-color CDM-TD metallic iodure discharge lamps and TLD 18-830 Philips neon lamps). A gradual change in the light intensity was introduced with three irradiation steps (0, 1/3, 2/3, 3/3) from 0 to 1200 μmol m^{-1}, to simulate natural daylight conditions. The light, temperature, CO_2 and RH conditions were regulated in the same way and monitored in all six phytotrons (Gullino et al. 2011).

The highly susceptible zucchini cv. Genovese (Furia Sementi, Parma, Italy) (trials 1-4) and leaf beet cv. Bietola verde da Taglio (Ortis, Emilia, Italy) (trials 5-8) plants were grown in 2 L plastic pots filled with a steamed (90°C for 30 minutes) mix of white peat: perlite, 80:20 v/v (Turco Silvestro, Albenga, Italy). The plants were kept at 22-24°C in a greenhouse until the phenological stage of the first true leaf was reached. One zucchini plant/pot and 25 leaf-beet plants/pot were used. Six pots per treatment were arranged in a completely randomized block design (one pot per block design) in each phytotron. A total of 18 and 24 pots were used in each replicated trial for Phoma leaf spot of leaf beet and powdery mildew of zucchini, respectively. The zucchini and leaf beet plants were transferred to phytotrons when the first true leaf stage had been reached, and were kept under six different combinations of temperature and CO_2: 1) 400-450 ppm CO_2, 18–22 °C; 2) 800-850 ppm CO_2, 18–22 °C; 3) 400-450 ppm CO_2, 22–26 °C, 4) 800-850 ppm CO_2, 22-26 °C, 5) 400-450 ppm CO_2, 26-30 °C; 6) 800-850 ppm CO_2, 26-30 °C (Table 1).

The artificial inoculations were carried out with a population of *P. xanthii* (Braun et al. 2000), obtained from diseased zucchini plants, while a strain of *P. betae*, isolated from diseased *B. vulgaris* subsp. *vulgaris* (Garibaldi et al. 2007), was prepared from a 10-day old culture of the fungus, grown on PDA at 24°C and 12 h of light/darkness. The artificial inoculation of the zucchini and leaf beet plants was carried out 24 h before the treatments by spraying a conidial suspension at $1x10^5$ conidia/ml. Inoculated and untreated plants were used as controls. After inoculation, the plants were covered with a transparent polyethylene film (50 microns thick) in a plastic container (100 ×100 ×50 cm) and incubated for 7 days in order to maintain very high RH and prolonged leaf wetness (Table 1).

Two chemicals, penconazole and sulphur, and one biocontrol agent were applied against the powdery mildew of zucchini, while two other fungicides, mancozeb and azoxystrobin, were used against the Phoma leaf spot of leaf beet. *Ampelomyces quisqualis* was used at 29 g/100L (AQ 10, CBC Europe, 58 % a.i.) as a commercial formulation and applied as a foliar spray at the suggested dosages recommended by the manufacturer; penconazole was applied at 4.06 g/100L (Topas10 EC, Syngenta Crop Protection S.p.A., Milano, Italy, 10.2 % a.i.), sulphur at 200 g/100 L (Tiovit Jet, Syngenta Crop Protection S.p.A., Milano, Italy, 80 % a.i), azoxystrobin at 18.6 g/100L (Ortiva, Syngenta Crop Protection S.p.A., Milano, Italy, 23.2 % a. i.) and mancozeb at 262.5 g/100L (Dithane DG, Neotec., Italy,75 % a.i.).

The chemical and biofungicide applications were carried out using a volume of 800 L ha^{-1}. The treatments were carried out 24 h after the artificial inoculation with the pathogen. One application was made for the chemical fungicides, while *A. quisqualis* was applied twice at a 7-day interval (Table 1).

The plants were checked weekly for disease development, and the percentage of infected leaves and the affected leaf area were evaluated, starting from the appearance of the first symptoms. Six to ten leaves of zucchini from each pot were examined

visually: the number of infected leaves was counted (disease incidence, *DI*), and the approximate leaf area affected by the disease was evaluated (disease severity, *DS*). *DI* and *DS* were estimated on 50 leaves/treatment for the leaf beet plants.

The severity of both plant diseases was evaluated using the following rating scale calculated as [∑(Number of leaves × *i* 0-5) / (total number of leaves recorded)] with *i* 0-5, where the index rating *i* value represents the midpoint of disease severity according to the following scale: 0=no symptoms, healthy plants; 1=1 to 10 % of affected leaf area (midpoint 5 %); 2=11 to 25 % of affected leaf area (midpoint 15 %); 3=25 to 50 % of affected leaf area (midpoint 35 %); 4=51 to 70% of affected leaf area (midpoint 60 %): 5=over 70% of affected leaf area (midpoint 75%).

The efficacy of different treatments in controlling the powdery mildew of zucchini and Phoma leaf spot on leaf beet (DI and *DS* controls) was calculated as:

$$\%\ Disease\ control\ (based\ either\ on\ DI\ or\ DS) = \frac{LS_i - LS_t}{LS_i} \times 100$$

where LS = Leaf spot index (percentage of leaves affected, for DI, percentage of leaf surface affected, for *DS*)

i = inoculated control (leaves artificially inoculated with the pathogen without treatments).

t = treatments.

The efficacy values were statistically analyzed by means of analysis of variance (ANOVA), in which the effects of temperature, the CO_2 concentration and product application, and their interactions on the disease development were tested. The analyses were conducted with SPSS software 22, and data from individual replicates are transformed are analyzed of the disease assessments derived from counting were arcsine-transformed before the statistical analysis. Since a preliminary ANOVA showed no significant effect of the repeated trials, the trials were considered as replicates. When the interaction of the tested factors was significant at $P<0.05$ and $P<0.1$, one-way ANOVA was carried out to evaluate the combined effect of the involved factors on percent disease control, on the basis of disease incidence, *DI*, or disease severity, *DS* (Table 2). Multiple comparisons of the effects of fixed factors were made by means of the Tukey–Kramer honestly significant difference (HSD) test.

In the four trials on the *P. xanthii*-zucchini pathosystem, the one-way Anova analysis showed as treatments ($P<0.0001$), CO_2 levels ($P=0.012$) and their interaction significantly ($P<0.001$) influenced percent powdery mildew control for *DS* (Table 2). An increase in CO_2 from 400-450 to 800-850 ppm significantly improved the powdery mildew control provided by *A. quisqualis*, by 8.6 % and 16.1% for *DI* and *DS*, respectively (Table 3). The efficacy of the penconazole and sulphur treatments was not affected by the higher CO_2 regimes that were tested (Table 3). Surprisingly, temperature was not a significant factor of influence on disease control as *DI* and *DS* ($P=0.192$; $P=0.215$, respectively), while the interaction with 'treatments' was

significant for *P*=0.052 and *P*=0.054, respectively (Table 2). *A.quisqualis* only provided a moderate disease control 12 days after the last application for all the tested temperature regimes (Table 4). However, its control was significantly improved for *DI* by 15.3% and for *DS* by 6%, when the temperatures were increased from 22-26°C to 26-30°C (Table 4). The disease control provided by the two fungicides was quite high, ranging from 85.0 to 88.9 % for penconazole and from 89.9 to 92.6 % for sulphur, and no significant effect of increased temperature and CO_2 on their efficacy was observed. A significant effect of the combined CO_2×temperature factors on powdery mildew control was observed (*P*=0.001 for *DI*) (Table 2). The disease control provided by *A. quisqualis* was significantly improved under an increased CO_2 concentration of 800-850 ppm at 26-30°C, that is, by 23.3% for *DI* and by 22.8 % for *DS*, while no differences in efficacy were observed for the chemical fungicides (Table 5).

In the *P. betae*-leaf beet pathosystem, the same analysis revealed that the temperature (*P*=0.002), CO_2 levels (*P*=0.011; *P*=0.029) and treatments (*P*<0.001, *P*<0.001) were significant for *DI* and *DS*. The Anova model also showed that the combinations of CO_2 × temperature, CO_2 × treatments, treatments × temperature, and treatments × CO_2 × temperature significantly influenced disease control, for both *DI* (*P* =0.053) and *DS* (*P*=0.035) (Table 2 and Table 6).Ten to twelve days after the last treatment, azoxystrobin and mancozeb provided significant disease control, which ranged from 42.6 to 64.7 % and 63.7 to 88.8 %, respectively (Table 6). Mancozeb provided a somewhat better disease control than azoxystrobin for standard CO_2 levels, that is, from 63.8 to 73.5 %, than from 58.6 to 62.9 %. The effectiveness of both fungicides was significantly improved at 22-26°C for higher CO_2 levels of 15.3% for *DI* and 20.2% for *DS* for mancozeb and 20.6 % for *DI* for azoxystrobin (Table 6).

The different temperature and CO_2 combinations tested under the present experimental conditions have provided clear evidence of a significant improvement of 23.3% for disease incidence and of 22.8 % for disease severity in powdery mildew control after the application of *A. quisqualis* at high CO_2 values at 26-30°C. However, little is known about the underlying mechanisms. An elevated CO_2 level may affect canopy size and reduce the nitrogen concentration in plants, thus providing a reduced susceptibility to powdery mildew (Thompson et al. 1993), or cause a delay in primary infection in some crops (Hibbenrd et al. 1996). Temperature may directly or indirectly affect the uptake, persistence and degradation of fungicides (Chen and McCarl 2001). Moreover, some BCAs are only active over a narrow temperature and relative humidity range. For instance, the antagonistic activity of many *Trichoderma* isolates has been found to be higher at 28°C (Gullino et al. 1987), and to be inhibited by low temperatures when applied against *Heterobasidium annosum* (Tronsmo and Dennis 1978) and *Botrytis cinerea* (Tronsmo 1980). The optimum temperatures for spore germination of *A. quisqualis* was found to be 25°C; germination decreased above 30°C and eventually stopped at 37°C (Kiss et al. 2004). In the present study, the increase in

temperature had a significant effect on powdery mildew control provided by *A. quisqualis*, that improved by 15% for temperatures of 26 to 30°C.

A change in disease progress has important implications on the effectiveness of protectant fungicides such as mancozeb and sulphur, which do not penetrate the cuticle and mainly exhibit preventative activity. At the same time, the morphological and physiological changes of plants grown under a high CO_2 level may affect the uptake and penetration of systemic fungicides, such as azoxystrobin and penconazole (Coakley et al. 1999). In the present study, the penconazole and sulphur treatments applied under a scenario of simulated CO_2 and temperature increases showed an efficacy in powdery mildew control that ranged from 85.0 to 88.9 % for penconazole and from 89.9 to 92.6 % for sulphur, and therefore resulted equally effective to that observed under 400-450 ppm of CO_2 level. On the contrary, the highest Phoma leaf spot control value has been provided by mancozeb, at 22-26°C under doubled CO_2 level, with a better efficacy of 20.2% for *DS* and 15.3% for *DI* than those observed at a 400-450 ppm CO_2 level. However, even though azoxystrobin has generally resulted to be less effective against Phoma leaf spot than mancozeb, its efficacy has resulted to have been improved by 20.6% at 22-26°C and 800 -850 ppm CO_2. It is known that fungicidal activity can be affected by such environmental conditions as temperature, relative humidity, CO_2, rainfall and soil properties. In addition, temperature may also affect disease progress, thus leading to the necessity of adapting the application frequency of the fungicides. An earlier occurrence in stem canker of oilseed rape epidemics has been blamed on climatic warming (Huang et al., 2007; Evans et al., 2008). However, early disease inoculum detection and identification are important to both stop epidemics and determine the spray timing for an optimal control.

The effectiveness of biocontrol agents may also vary according to the environmental conditions. If biocontrol agents are to be used successfully as a pest management component, environmental conditions, such as the combined effect of temperature and CO_2, should be considered.

This study suggests that the activity of *A. quisqualis* against zucchini powdery mildew is generally improved in a climate change scenario. Moreover, the effectiveness of both mancozeb and azoxystrobin against Phoma leaf spot is affected by high levels of CO_2 at 22-26°C while, penconazole and sulphur treatments, applied under a simulated CO_2 and temperature increase scenario resulted to be equally effective to the efficacy observed under standard conditions.

Further studies would be useful to better understand the mechanisms implicated in the differences on effectiveness provided by fungicides and BCA tested in phytotrons under increased temperature and CO_2 combination, also with pathosystems and control measures.

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Table 1 Main information on the conducted trials and operations, starting from the transfer of plants to the phytotrons. Host plants and diseases are indicated in first row of the table

Main information	Zucchini/powdery mildew trials					Leaf beet/*Phom*
	1[a]	2	3	4	5	6
Age of plants from sowing until transfer to the phytotron	14 days	8 days	9 days	8 days	14 days	7 days
Artificial inoculations with the pathogen	T7	T7	T7	T7	T7	T7
1st treatment	T8	T 8	T8	T8	T8	T8
2nd treatment[b]	T15	T16	T14	T15	T15	-
Appearance of the first symptoms	T15	T13	T18	T12	T12	T13
Final Assessment	T25	T23	T21	T18	T18	T20

[a] The start of the trials corresponds to the transfer of the zucchini and leaf beet plants to the phytotrons: Trial 1 - 25/06/2015; Trial 2 - 14/07/2015; Trial 3 - 07/08/2015; Trial 4 - 29/09/2015; Trial 5 - 25/06/2015; Trial 6 - 14/07/2015; Trial 7 - 31/08/2015; Trial 8 - 6/10/2015

[b] One application was made for the chemical fungicides, while *A. quisqualis* was applied twice at a 7-day interval

Table 2 Significance values for the CO_2, Treatments and Temperature factors and their interactions on the Disease incidence (*DI*) and Disease severity (*DS*) reduction of zucchini powdery mildew and leaf beet Phoma leaf spot

Fixed factor	Zucchini/powdery mildew trials		Leaf beet/*Phoma* leaf spot trials	
	Sign. (% reduction *DI*)	Sign. (% reduction *DS*)	Sign. (% reduction *DI*)	Sign. (% reduction *DS*)
Trial	0.551	0.800	0.325	0.169
CO_2 concentration	0.076[b]	0.012[a]	0.011[a]	0.029[a]
Treatments	<0.0001[a]	<0.0001[a]	<0.0001[a]	<0.0001[a]
Temperature	0.192	0.215	0.002[a]	0.057 [b]
CO_2 × Treatment	0.022[a]	<0.0001[a]	0.042[a]	0.020[a]
CO_2 × Temperature	0.001[a]	0.091[b]	0.001[a]	0.127
Treatment × Temperature	0.052[a]	0.054[a]	0.013[a]	0.237
CO_2 × temperature × treatment	0.759	0.366	0.053	0.035

[a] Significant effect at $P<0.05$

[b] Significant effect at $P<0.1$

Table 3 Effect of different treatments (biological and chemical) at two CO_2 concentrations (400-450 and 800-850 ppm) on zucchini powdery mildew expressed as percent disease control, based on Disease incidence (*DI*) and Disease severity (*DS*) reduction compared to the inoculated untreated control plots[b]

Treatment	CO_2 (ppm)	*DI* reduction			*DS* reduction		
A.quisqualis	400-450	31.3	±3.2	d[a]	42.6	±3.1	c
A.quisqualis	800-850	41.7	±3.1	c	58.7	±3.2	b
Penconazole	400-450	84.4	±2.7	b	92.9	±1.8	a
Penconazole	800-850	88.8	±2.7	ab	93.0	±2.1	a
Sulphur	400-450	93.0	±1.8	a	97.4	±0.8	a
Sulphur	800-850	89.4	±2.3	ab	94.9	±1.4	a

[a] Values with a common letter in the same column are not significantly different, according to Tukey's Test ($P<0.05$). Values are the mean of the experimental replicates ± standard error

[b] Average *DI* and *DS* for the inoculated untreated control plots at the end of the trials at temperatures of 18–22, 22-26 and 26-30 °C under CO_2 at 400-450 ppm corresponded to: *DI* of 58.2, 69.8 and 60.9 and *DS* of 19.3, 33.6 and 25.2. For CO_2 at 800-850 ppm corresponded to: *DI* of 63.2, 70.2 and 52.5 and *DS* of 28.9, 28.4 and 19.6, respectively

Table 4 Effect of different treatments (biological and chemical) at different temperatures (18-22, 22-26 and 26-30 °C) on zucchini powdery mildew, expressed as percent disease control, based on Disease incidence (*DI*) and Disease severity (*DS*) reduction compared to the inoculated untreated control plots[b]

Treatment	Temperature (°C)	*DI* reduction	*DS* reduction
A.quisqualis	18-22	36.5 ±3.5 bc[a]	54.3 ±3.9 b
A.quisqualis	22-26	29.1 ±2.8 c	48.0 ±3.7 c
A.quisqualis	26-30	44.0 ±4.2 b	54.1 ±4.2 b
Penconazole	18-22	85.9 ±4.1 a	90.1 ±3.4 a
Penconazole	22-26	88.9 ±2.5 a	94.6 ±1.7 a
Penconazole	26-30	85.0 ±3.3 a	94.2 ±1.7 a
Sulphur	18-22	91.0 ±2.6 a	96.3 ±1.5 a
Sulphur	22-26	89.9 ±2.5 a	95.5 ±1.6 a
Sulphur	26-30	92.6 ±2.4 a	96.8 ±1.2 a

[a] Values with a common letter in the same column are not significantly different, according to Tukey's Test ($P<0.05$). Values are the mean of the experimental replicates ± standard error

[b] Average *DI* and *DS* for the inoculated untreated control plots at the end of the trials at temperatures of 18–22, 22-26 and 26-30 °C under CO_2 at 400-450 ppm corresponded to: *DI* of 58.2, 69.8 and 60.9 and *DS* of 19.3, 33.6 and 25.2. For CO_2 at 800-850 ppm corresponded to: *DI* of 63.2, 70.2 and 52.5 and *DS* of 28.9, 28.4 and 19.6, respectively

Table 5 Efficacy of fungicide treatments at different CO_2 concentrations (400-450 and 800-850 CO_2) and temperatures (18-22, 22-26 and 26-30 °C) on zucchini powdery mildew, expressed as percent disease control, based on Disease incidence (*DI*) and Disease severity (*DS*) reduction compared to the inoculated untreated control plots[b]

Treatment	Temperature (°C)	CO_2 (ppm)	*DI* reduction	*DS* reduction
A. quisqualis	18-22	400-450	34.6 ±5.8 d[a]	46.2 ±5.8 d
A. quisqualis	18-22	800-850	38.3 ±3.9 d	62.5 ±4.8 bc
A.quisqualis	22-26	400-450	27.0 ±3.7 d	38.8 ±5.1 d
A.quisqualis	22-26	800-850	31.2 ±4.2 d	48.0 ±5.5 cd
A.quisqualis	26-30	400-450	32.4 ±4.6 d	42.7 ±5.3 d
A.quisqualis	26-30	800-850	55.7 ±6.2 c	65.5 ±5.8 b
Penconazole	18-22	400-450	88.8 ±4.8 ab	94.2 ±3.6 a
Penconazole	18-22	800-850	82.9 ±7.0 ab	86.0 ±5.7 a
Penconazole	22-26	400-450	85.2 ±4.0 ab	91.7 ±3.1 a
Penconazole	22-26	800-850	92.6 ±2.7 ab	97.5 ±1.1 a
Penconazole	26-30	400-450	79.3 ±5.4 b	92.8 ±2.6 a
Penconazole	26-30	800-850	90.8 ±3.6 ab	95.6 ±2.3 a
Sulphur	18-22	400-450	98.4 ±1.6 a	99.7 ±0.3 a
Sulphur	18-22	800-850	83.7 ±4.6 ab	92.8 ±2.9 a
Sulphur	22-26	400-450	91.6 ±2.8 ab	97.9 ±0.8 a
Sulphur	22-26	800-850	88.1 ±4.2 ab	93.0 ±3.0 a
Sulphur	26-30	400-450	88.9 ±2.3 ab	94.7 ±0.7 a
Sulphur	26-30	800-850	96.3 ±4.0 ab	99.0 ±2.2 a

[a] Values with a common letter in the same column are not significantly different, according to Tukey's Test ($P<0.05$).

Values are the mean of the experimental replicates ± standard error

[b] Average *DI* and *DS* for the inoculated untreated control plots at the end of the trials at temperatures of 18–22, 22-26 and 26-30 °C under CO_2 at 400-450 ppm corresponded to: *DI* of 58.2, 69.8 and 60.9 and *DS* of 19.3, 33.6 and 25.2. For CO_2 at 800-850 ppm corresponded to: *DI* of 63.2, 70.2 and 52.5 and *DS* of 28.9, 28.4 and 19.6, respectively

Table 6 Efficacy of fungicide treatments at different CO_2 concentrations (400-450 and 800-850 ppm) and temperatures (18-22, 22-26 and 26-30 °C) against *Phoma betae* on leaf beet, expressed as percent disease control, based on Disease incidence (*DI*) and Disease severity (*DS*) reduction compared to the inoculated untreated control plots[b]

Treatment	Temperature (°C)	CO_2 (ppm)	*DI* reduction			*DS* reduction		
Mancozeb	18-22	400-450	74.9	±4.2	a-c[a]	73.5	±6.4	ab
Mancozeb	18-22	800-850	86.2	±2.9	ab	73.9	±8.1	ab
Mancozeb	22-26	400-450	73.5	±3.6	b-d	65.2	±5.0	ab
Mancozeb	22-26	800-850	88.8	±2.1	a	85.4	±6.6	a
Mancozeb	26-30	400-450	63.7	±5.1	c-e	63.8	±6.5	ab
Mancozeb	26-30	800-850	69.3	±4.3	cd	69.1	±4.6	ab
Azoxystrobin	18-22	400-450	64.8	±3.6	cd	58.6	±6.9	b
Azoxystrobin	18-22	800-850	59.2	±3.9	de	64.4	±6.9	ab
Azoxystrobin	22-26	400-450	42.6	±5.3	f	58.9	±4.7	b
Azoxystrobin	22-26	800-850	63.2	±4.5	c-e	68.5	±6.2	ab
Azoxystrobin	26-30	400-450	60.8	±3.9	c-e	62.9	±7.5	b
Azoxystrobin	26-30	800-850	49.5	±4.3	ef	53.9	±7.6	b

[a] Values with a common letter in the same column are not significantly different, according to Tukey's Test ($P<0.05$). Values are the mean of the experimental replicates ± standard error

[b]Average *DI* and *DS* for the inoculated untreated control plots at the end of the trials at temperatures of 18–22, 22-26 and 26-30 °C under CO_2 at 400-450 ppm corresponded to: *DI* of 37.9, 44.3 and 39.5 and *DS* of 17.0, 20.2 and 20.6. Under CO_2 at 800-850 ppm corresponded to: *DI* of 42.6, 50.3 and 49.6 and *DS* of 18.6, 20.4 and 20.5, respectively