Effect of elevated atmospheric CO2 and temperature increases on the severity of basil downy mildew caused by Peronospora belbahrii under phytotron conditions.

Phytotron to simulate climate changes on basil downy mildew

Effect of elevated atmospheric CO_2 and temperature increases on the severity of basil downy mildew caused by *Peronospora belbahrii* under phytotron conditions

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Abstract

Three experimental trials have been carried out on the basil *(Ocimum basilicum)* - downy mildew *(Peronospora belbahrii*) pathosystem, under phytotron conditions, to evaluate the effect of simulated elevated atmospheric CO_2 concentrations and temperatures as well as that of their interaction. Six CO_2 and temperature combinations were tested to establish their effect on disease development. The photosynthetic efficiency (PI) and Chlorophyll Content Index (CCI) of the basil plants were monitored throughout the trials. Disease incidence ranged from 26.9 to 80.7% in the different trials, under standard conditions (18-22°C and 400-450 ppm of CO_2, while disease severity varied between 10.2 and 20.2%. In the same temperature regime, a doubled level of CO_2 caused a significant increase in both disease incidence and severity. When temperatures ranged between 22 and 26 °C, the effect of CO_2 increased disease incidence, but severity was less evident. At the highest temperatures tested, that is 26-30°C, which are not favorable for downy mildew development, the increase in CO_2 had virtually no effect on disease incidence or severity. By considering the PI value, of inoculated and non inoculated plants after an initial increased at time 8, with the exception at 26-30 °C for both CO2 conditions, a decreasing trend of PI was observed particularly pronounced at high CO2 levels. In the same way as for disease development, lower values ($P < 0.05$) were recorded for the inoculated plants at the end of the experiment at 18-22 °C for both CO_2 concentrations, while only at 850 ppm of CO_2 were lower values recorded at 22-26° C. As expected, the non-inoculated plants showed higher photosynthetic efficiency than the inoculated plants. Similar trends were also observed for the CCI, thus confirming that downy mildew incidence and severity, which in particular caused foliar damage at high CO_2 concentrations, led to a decrease in the physiological performances.

Key words: climate changes; physiological parameters; *Ocimum basilicum*

Introduction

Research conducted over the past decades has shown how the climate has changed and has been predicted to change continuously in the future (IPCC2014). The atmospheric CO_2 concentration is expected to reach 730 to 1020 ppm by 2100, compared to the 400.26 forecast for 2015 (Mauna Loa Observatory), as a consequence of human activities, including the combustion of fossil fuels (coal, natural gas, and oil) for energy and transportation, as well as for industrial processes and land-use (IPCC 2014; Meehl et al., 2007; Sanderson et al., 2011). For the past ten years (2005 - 2014), the average annual rate of increase has been 2.11 parts per million (ppm). An increase in the atmospheric concentration of CO_2 and other greenhouse gases will lead to an increase of between 1.8 and 4°C in the global mean temperature (Meehl et al., 2007), and consequently these higher CO_2 concentrations should be considered together with the increased temperatures. The number of cold days and nights in the Northern Hemisphere has decreased globally/throughout the world, and, from 1983 to 2012, were the warmest in the last 1,400 years (IPCC2013). Increases in CO_2 and temperature induce complex effects on plant pathosystems.

Since both CO_2 and temperature are key variables that can affect plants and their diseases, climate changes are influencing plant growth, plant diseases and, consequently, the global food supply (Chakraborty and Newton, 2011).

. Plant gene expression, plant physiology and population biology are all being influenced by these changes (Garrett et al. 2006), due to increases in the leaf area, leaf thickness, canopy size and density, as well as in the stomatal density (Coakley et al., 1999). Apart from pathogen fecundity, plant growth and virulence (Chakraborty, 2005; Luck et al., 2011) may also be affected directly by climate changes (Juroszek and Von Tiedemann, 2013).

Different approaches have been used to study the effect of increased temperature and CO_2 on diseases, including laboratory and field studies, as well as modeling-based assessments (Salinari et

al., 2006). Phytotron-based studies permit a precise control of environmental parameters, such us temperature, relative humidity, air, light, CO_2 concentration, air speed, leaf temperature and wetness (Gullino et al., 2011). It is impossible to achieve such a degree of control under natural field conditions.

Over the last decade, phytotrons have been used to study the effects of CO_2 enrichment and temperature increases on infection rates for several pathosystems (Ainsworth and Long 2005; Chakraborty 2005; Garrett et al. 2006; Grünzweig,2011; Pugliese et al. 2012 a, b; Ferrocino et al. 2013; Singh et al., 2014).

Foliar pathogens are influenced to a great extent by environmental conditions. Among the various foliar diseases, downy mildew is expected to become more problematic due to the foreseen temperature increases, which could directly or indirectly affect both the host and the pathogen (Salinari et al., 2006). Warmer temperatures and a reduction in the length of humid weather periods have reduced the severity of late blight potato disease (Schaap et al. 2011). On the other hand, , the increase in severity and the earlier occurrence in *Phytophthora infestans* epidemics observed in potatoes in Finland have been blamed on climatic warming and a lack of rotation (Hannukkala et al., 2007). Basil downy mildew causes severe losses of basil, wherever this crop is grown, and affects the production of this herb for its fresh consumption and pesto sauce production. The disease was first reported in Italy in 2003 (Garibaldi et al., 2004) under high relative humidity (RH) and warm temperature conditions, which are known to favor disease development (Garibaldi et al., 2007).

In this study, the basil (*Ocimum basilicum*) - downy mildew (*Peronospora belbahrii*) pathosystem was chosen to evaluate the effect of simulated elevated atmospheric CO_2 concentrations and temperatures, as well as that of their interaction, under phytotron conditions. Six combinations of CO_2 and temperatures were tested to establish their effect on disease development and on the physiological parameters in order to observe the plants sensing and response to rising CO_2

concentrations and temperatures on the photosynthetic apparatus, using the chlorophyll content (CCI) and photosynthetic efficiency (PI) indices.

Material and methods

Plant material

About 30 seeds of basil belonging to the Genovese 'Italiano classico' selection (Pagano) were sown in 2 L plastic pots filled with a steamed (90°C for 30 minutes) white peat:perlite mix, 80:20 v/v (Turco Silvestro, Albenga, Italy). The same seed lot was used in the three trials. The seed lot was naturally infected with *P. belbahrii* at a level of approximately 0.6 infected seeds out of 1,000, according to the experimental protocol reported in Garibaldi *et al*., (2004). Twenty-five basil plants were used per pot. Before starting the experiments, the plants were kept at 22-24°C in a greenhouse until the phenological stage of the first true leaf was reached.

A total of six pot replicates (one pot = experimental unit with 25 plants/pot for a total of 6 pots/ phytotron) were examined for each of the six experimental conditions. The pots were rotated weekly within the phytotron to avoid chamber effects. At the end of each trial, each phytotron was cleaned carefully.

Artificial inoculation with *Peronospora belbahrii*

The inoculum was produced from one population of *P. belbahrii*, which was obtained from diseased basil plants on a commercial farm in Piedmont (Northern Italy) and maintained on basil plants. A suspension of viable sporangia of the pathogen was prepared by shaking th infected basil leaves in 100 ml of sterile water containing 2 µl of Tween 20; the suspension was adjusted with a

haemocytometer to $1x10^5$ conidia ml^{-1}. Healthy basil plants were acclimated for seven days in each phytotron under the above controlled environmental condition, before the inoculation of 1 ml of suspension/pot, which was applied to the basil plants using a hand-held sprayer (10 ml capacity). After inoculation, the pots were placed on a plastic support (100x100x50 cm) and were covered with a transparent polyethylene film (50 microns thick) for 7 days in order to maintain the high RH conditions. The dates of the artificial inoculations in the different trials and, the dates of the operational events of each trial are reported in Table 1.

Experimental conditions

The effects of elevated CO_2 and temperature were studied in six physically and electronically separated phytotrons, with 2 m wide x 2 m long x 2.5 m high internal dimensions (Gullino et al., 2011). A 14/10-h day/night photoperiod was provided by two lighting systems (master-color CDM-TD metallic iodure discharge lamps and TLD 18-830 Philips neon lamps. Agradual change in the light intensity regime, resulting from three irradiance steps (0, 1/3, 2/3, 3/3) from 0 to 1200 μmol m^{-2} s^{-1},was undertaken/introduced to simulate natural daylight. . Each phytotron was regulated in the same way and maintained at high relative humidity, close to 85-95%.

The environmental parameters (light, temperature, humidity and CO_2) inside the phytotrons were monitored continuously and kept constant (Gullino et al., 2011).

The basil plants were maintained/kept in the phytotrons 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. In each trial, one environmental combination corresponded to one phytotron. The phytotrons were randomized by changing the environmental conditions and combinations from one trial to another. Three experimental trials were carried out as separate studies in 2014, under completely controlled environmental conditions in the phytotrons (Table 1).

149

150 Disease assessment

151 The plants were checked weekly for disease development, which was considered to have started
152 with the appearance of the first symptoms, that is, leaf chlorosis. Fifty randomly chosen basil
153 leaves from each pot were examined visually: the number of infected leaves was counted. Data
154 were expressed as the percentage of leaves showing infection (disease incidence), and the estimated
155 leaf area affected by the disease was evaluated (disease severity). Disease severity was evaluated
156 using a disease rating scale calculated as [∑(n° leaves × x 0-5) / (total leaves recorded)] with x 0-5
157 corresponding to the value reported: 0=no symptoms, healthy plants; 1=1 to 30% affected leaf area
158 (midpoint 15%); 2=31 to 50% affected leaf area (midpoint 40%); 3=51 to 70% affected leaf area
159 (midpoint 60%); 4=71 to 90% affected leaf area (midpoint 80%): 5=over 90% affected leaf area
160 (midpoint 95%).

161

162 Physiological measurements

163 In order to observe the effects of the climate change conditions on the leaf physiological activity of
164 the basil plants (healthy and affected by *P. belbahrii*), the photosynthetic efficiency and chlorophyll
165 content of the plants were monitored as described hereafter. Measurements were performed
166 according to the experimental protocol reported in Pugliese *et al.*, (2010), with only minor
167 modifications.

168 The chlorophyll content index (CCI) was measured using a SPAD 502 chlorophyll meter (CCM-
169 200, Opti-Sciences, Inc., Hudson, NH, USA), which determined the relative amount of chlorophyll
170 in the leaf by measuring the absorbance in the red and near-infrared regions (650 and 940 nm,
171 respectively). Chlorophyll meter readings were taken of the second or third leaves (fully

developed) of each basil plant, from the top, on ten randomly selected plants (one leaf/plant) at time 0 and at 8, 16 and 23 days (end of experiment) for the inoculated and non-inoculated basil plants. Photosynthetic efficiency measurements were performed on five randomly selected leaves, using a portable continuous-excitation type fluorimeter (Handy-PEA, Hansatech Instruments ltd, Norefolk, UK), according to the manufacturer's instructions, at time 0 and from day 8 every seven days, up to the end of the trial for both the inoculated and non-inoculated plants.

Statistical analysis

All the analyses were conducted using the Superior Performing Software System SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Levene's Test was used to assess the homoscedasticity of Variance. Two-way Anova was used to investigate the effect of each factor (CO_2 and temperature), and their interactions, on disease incidence and severity caused by *P. belbahrii* on basil, and the means were calculated according to Tukey's HSD test ($P < 0.05$). The average disease assessment values, derived from counting, were arcsine-transformed before the statistical analysis was performed to make the data closer to a normal distribution. The back-transformed mean values are shown in Table 2.

Results

Effect on disease development

From a comparison of the trials, it has emerged that the assumptions of normality and homoscedasticity were violated for disease incidence ($P < 0.05$), but were confirmed for disease severity. The disease severity data were combined and analyzed as the average of three trials, using the ANOVA two-way analysis of variance, while the Tukey post- hoc test was used to compare all

196 the possible combinations of group differences (Figure 1). The data, in the case of disease
197 incidence, have been reported separately for each trial (Table 2).
198 The two-way analysis of variance analysis confirmed that CO_2 and temperature, as well as the
199 interactions between these factors, significantly influenced the disease incidence ($P < 0.001$) and
200 severity ($P < 0.001$) caused by *P. belbahrii* in all the trials (Figure 1, Table 2).
201 In the presence of the standard conditions (18-22°C and 400-450 ppm of CO_2), the disease index
202 varied from 26.9 to 80.7 over the different trials, while disease severity varied between 10.2 and
203 20.2 (Table 2). A doubled level of CO_2 caused a significant and notable increase in both disease
204 incidence and severity for the same temperature ranges.
205 When the temperatures ranged between 26 and 30 °C, the effect of CO_2 on increasing disease
206 incidence and severity was less evident. At the highest temperatures tested, that is, at 26-30°C, the
207 increase in CO_2 caused a not always significant increase in disease incidence and severity (Table
208 3).
209
210 Effect on the leaf physiological measurements

211 In the inoculated and non-inoculated plants, PI, after an initial increase in the values at time 8, with
212 the exception at 26-30 °C for both CO_2 conditions, a decreasing trend was observed particularly
213 pronounced and statistically significant at high CO_2 levels at 18-22 °C and 22-26 °C at the end of
214 experiment (Table 3). In agreement with disease development (Table 2), lower values ($P < 0.05$)
215 were recorded for the inoculated plants at the end of the experiment, at 18-22 °C for both CO_2
216 states, while only 850 ppm CO_2 was recoded at 22-26° C. The non-inoculated plants showed
217 higher photosynthetic efficiency than the inoculated plants.
218 Similar trends were also observed for the CCI measurements, with the exception of those measured
219 at a high temperature. Higher CCI values were obtained for higher CO_2 conditions (Table 4), where
220 no influence on chlorophyll content was observed, compared with the non-inoculated basil plants.

Overall, the physiological measurements have confirmed that the disease development, which in particular caused foliar damage at high CO_2 concentrations, led to a decrease in the physiological performances.

Discussion

The connection between climatic changes and plant disease severity in several pathosystems has received more and more attention (Pautasso et al., 2012; Pangga et al., 2013). Phytotrons have frequently been used to simulate a climate change scenario, because they make it possible to maintain total of the environmental conditions and thus to provide reproducible data, while avoiding the high risk of fluctuations due to other factors that can be observed in natural conditions. The present work was carried out in phytotrons in order to evaluate the effect of increased carbon dioxide concentrations, and increased temperature, as well as the combination of both these factors on basil downy mildew, in highly controlled environments. A good disease level was reached in the three trials, thus making it possible to evaluate the effect of the different quantities of CO_2 and temperature increases.

Two-way Anova has shown that the temperature and CO_2 of the six simulated environmental conditions had a significant influence on the severity of basil downy mildew. A double concentration of CO_2 caused a significant increase in downy mildew severity, in particular at temperatures of 18-22 °C and 22-26°C. The effect of CO_2 on disease severity development was observed at 26-30°C, although it was not always significant (Figure 1). Although elevated CO_2 alone had a significant effect on downy mildew severity, the increase in temperature also had a significant effect on disease development. At average temperatures of 22 °C, which are favorable for disease development (Garibaldi et al., 2007), elevated CO_2 may favor the growth of the pathogen by increasing the sugar supply (Horsfall and Dimond, 1957; Stitt and Krapp, 1999; Mahatma et al., 2009).

246 The direct effect of increased CO_2 values on plant diseases has been investigated less than the effect
247 of temperature increases. The effect of elevated CO_2 on foliar fungal disease severity may depend
248 on the photosynthetic pathway of a plant, and disease severity could in particular be increased
249 through a decrease in water stress, which can increase fungal sporulation. In general, most of the
250 conducted researches have been carried out on cereal crops, which responded differently, according
251 to their sensitivity to elevated CO_2. For example, von Tiedemann and Firsching (2000) found that
252 nitrogen-fixing legumes were more sensitive to elevated CO_2 and consequently to disease, while
253 elevated CO_2 did not affect the leaf rust disease of wheat. Grünzweig (2011) showed that high CO_2
254 could affect the susceptibility of *Onobrychis crista-galli* to powdery mildew. In other cases, it has
255 been shown that elevated CO_2 did not influence the disease of zucchini powdery mildew (Pugliese
256 et al., 2012b) or was correlated to an increase in temperatures for powdery mildew on grapevine
257 (Pugliese et al., 2010) and to the black spot disease of basil (Pugliese et al., 2012a). In he present
258 study, the impact of elevated CO_2 on basil downy mildew severity was much more pronounced than
259 on the physiological parameters.
260 Environmental stimuli, including elevated carbon dioxide levels and temperatures, regulate
261 physiological performances and hence host-pathogen interaction. It is well known that global
262 changes are modelled by the connection between atmosphere, land, water, ice and vegetation.
263 Moreover, human activities, such as deforestation and increased CO_2 emissions, have accelerated
264 some of these changes. High CO_2 could cause changes in the anatomy and morphology of the host
265 plant, and thus influence resistance, host-pathogen interaction and disease epidemiology (Hartley et
266 al., 2000; Pangga et al., 2004). Two trends could be designed for plant diseases that deteriorate with
267 rises in CO_2 and temperatures, i) the enlarged plant canopy offers more infection sites, and some
268 fungal pathogens can produce more spores (Idso and Idso, 1994; Chakraborty et al., 2000); ii) an
269 increased fungal fecundity and the increased number of spores trapped by the enlarged canopy lead
270 to increased lesions at high CO_2 (Pangga et al., 2004). This evidence reflects the results that have
271 been reported in this study. It is often stated that growth at high carbon dioxide levels stimulates

assimilation rates (Nowak et al., 2004; Ainsworth and Long, 2005). However, as reviewed/pointed out by Ainsworth and Rogers (2007), the maximum carboxylation rate and electron transport in C3 species are significantly reduced at elevated CO_2 and temperature conditions. This result is in agreement with the photosynthetic efficiency (PI) (Table 3) measurements reported here both for inoculated and non-inoculated basil plants. Moreover, the CO_2 sensing mechanism in guard cells is still unknown and open to debate. Further free-air CO_2 enrichment (FACE) experiments are needed to obtain more detailed knowledge on this key phenomenon (Ainsworth and Rogers, 2007). Similar trends of PI were here observed in the leaf chlorophyll content, which is an indicator of photosynthetic activity and chlorophyll stability for the conjugation of assimilates. SPAD chlorophyll meters are frequently used for the quantitative measurement of foliar damage provoked by different biotic and abiotic stresses (Bijanzadeh and Emam, 2010; Pugliese et al., 2010). The measurement of the photosynthetic efficiency and the relative amount of chlorophyll in the leaf is a rapid and effective way of establishing the healthy status of the photosynthetic machinery. A significant decrease in the total chlorophyll content in susceptible genotypes of *Pennisetum glaucum* affected by *Sclerospora graminicola* has been reported (Mahatma et al., 2009).

Considering the rise in CO_2 levels, it can be assumed that such changes could affect the severity of basil downy mildew in the same way as that of other downy mildew pathogens (Salinari et al., 2006). Carbon dioxide enrichment is another technique that is used to increase both yield and profit (Huckstadt et al., 2013), but is not recommended for the cultivation of basil, due to the fact it can intensify downy mildew and other diseases; as yet,, this practice has not been applied in Italy.

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396

397 Table 1 Main information on the three trials and dates of operations carried out starting from transfer of
398 plants in phytotrons

	Trial 1	Trial 2	Trial 3
Plant age (days from sowing to phytotron transfer)	18	11	17
Transfer of plants in phytotron conditions [a]	T0	T0	T0
Artificial inoculations with *P. belbahrii*	T7	T8	T7
Physiological measurements		T8	T9
		T17	T18
Final disease assessment	T14	T22	T18
End of the trial	T14	T22	T18

399 [a] Start of the trial corresponding to time 0 in Trial 1: 18/03/2014, Trial 2 :7/04/2014 and Trial 3 2/05/2015.

400

401

402 Table 2. Effect of different CO_2 and temperature regimes on the development of *P. belbahrii* on basil, cv.
403 Italiano, artificially inoculated, expressed as percent of infected leaves (Disease incidence, DI)

Temperature °C[a]	CO_2 ppm	DI at the end of trial					
		1		2		3	
18-22	400-450	26.9±6.7	ab[b]	35.3±5.8	a	80.7±6.3	ab
18-22	800-850	81.3±16.4	c	88.0±9.3	c	93.0±4.5	c
22-26	400-450	11.3±3.3	a	76.0±7.0	b	81.3±6.7	ab
22-26	800-850	70.7±12.4	c	68.3±9.4	b	91.7±2.3	bc
26-30	400-450	5.7±2.0	a	38.0±9.3	a	79.5±8.3	a
26-30	800-850	11.8±4.7	a	47.7±7.9	a	81.3±7.2	ab

404 [a] Minimum and Maximum air temperature in each phytotron.

405 [b]Means of the same column, followed by the same letter, do not significantly differ following
406 Tukey'sHSD test ($P < 0.05$).

407

Table 3 Photosynthetic efficiency (PI. Absorbance, ABS) of inoculated and non inoculated control basil plants following incubation in phytotrons

Trial condition	PI (ABS ± SD)	Phytotron at 18-22 °C with CO2 at						Phytotron at 22–26°C with CO2 at						Phytotron at 26–30°C with CO2 at					
	Days of placing	400-450 ppm			800-850 ppm			400-450 ppm			800-850 ppm			400-450 ppm			800-850 ppm		
Before transfer basil in phytotron on	0[a]	28.4±	7.6	d-i[b]	28.4 ±	7.6	d-i	28.4±	7.6	d-i	28.4±	7.6	d-i	28.4±	7.6	d-i	28.4±	7.6	d-i
11-17 days old plants Inoculated	8	31.7±	15.6	d-i	39.7 ±	7.8	ij	37.9±	5.8	g-j	32.7±	12.7	d-j	28.2±	8.6	d-i	22.6±	5.9	b-g
	16	30.9±	15.4	d-i	26.4 ±	16.7	c-i	31.4±	3.1	d-i	29.1±	10.0	d-i	40.6±	3.9	ij	19.2±	15.8	b-e
	End of experiment	12.0±	2.3	a-c	9.0 ±	8.8	ab	28.1±	4.9	d-i	3.4±	2.5	a	40.0±	7.1	ij	18.5±	11.1	b-d
Non inoculated	8	37.1±	2.7	g-j	33.0 ±	6.1	d-j	35.9±	5.5	f-j	40.5±	13.8	ij	28.7±	8.7	d-i	23.3±	4.6	b-h
	16	35.0±	16.2	e-j	47.7 ±	11.5	jk	36.9±	9.6	f-j	34.7±	6.0	e-j	41.7±	5.1	ij	41.5±	13.5	ij
	End of experiment	29.4±	4.0	d-i	39.0 ±	27.7	h-j	40.1±	15.5	hi	21.1±	12.7	b-f	57.3±	24.6	k	28.9±	9.9	d-i

[a] Immediately before placing plants in phytotrons.

[b] Means followed by the same letter, do not significantly differ following Tukey'sHSD test ($P<0.05$).

Table 4 Chlorophyll Content Index (CCI. °SPAD) of inoculated and non inoculated basil plants following incubation in phytotrons

Trial condition	CCI (°SPAD± SD)	Phytotron at 18-22 °C with CO2 at						Phytotron at 22–26°C with CO2 at						Phytotron at 26–30°C with CO2 at					
	Days of placing	400-450 ppm			800-850 ppm			400-450 ppm			800-850 ppm			400-450 ppm			800-850 ppm		
Before transfer basil in phytotron on 11-17 days old plants	0[a]	7.3±	0.8	a-h[b]	7.3±	0.8	a-h	7.3±	0.8	a-h	7.3±	0.8	a-h	7.3±	0.8	a-h	7.3±	0.8	a-k
Inoculated	8	10.0±	2.8	c-k	6.9±	4.4	a-f	7.0±	2.7	a-g	9.5±	1.4	b-l	8.0±	3.9	a-i	9.1±	0.8	b-k
	16	6.0±	5.0	a-d	12.7±	2.4	h-l	8.5±	5.0	a-j	13.7±	2.6	j-l	12.9±	1.4	h-l	13.3±	1.0	i-l
	End of experiment	4.7±	1.5	a-c	4.3±	0.6	ab	13.0±	2.4	i-l	3.6±	3.2	a	6.3±	0.9	a-e	11.9±	2.6	e-k
Non inoculated	8	9.7±	4.1	b-k	12.6±	2.1	g-l	7.9±	4.2	a-i	9.6±	2.4	b-k	9.8±	1.4	b-k	9.4±	0.5	b-k
	16	17.5±	2.1	l	14.5±	2.7	kl	10.8±	1.5	d-k	12.9±	0.8	h-l	11.5±	0.9	d-k	14.6±	3.0	kl
	End of experiment	10.9±	1.7	d-k	11.7±	7.5	e-k	12.1±	2.5	f-l	7.7±	0.9	a-i	6.4±	1.7	a-e	10.9±	1.1	c-k

[a] Immediately before placing plants in phytotrons.

[b]Means followed by the same letter, do not significantly differ following Tukey'sHSD test ($P<0.05$).

Figure 1. Effect of different CO_2 and temperature regimes on the development of *P. belbahrii* on basil, cv. Italiano, artificially inoculated, expressed as percent of affected leaf area (Disease severity, DS)