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1 Phytotron to simulate climate changes on basil downy mildew

2

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

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24

25 **Abstract**

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

47

48

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

50 **Introduction**

51

52 Research conducted over the past decades has shown how the climate has changed and has been
53 predicted to change continuously in the future (IPCC2014). The atmospheric CO₂ concentration is
54 expected to reach 730 to 1020 ppm by 2100, compared to the 400.26 forecast for 2015 (Mauna Loa
55 Observatory), as a consequence of human activities, including the combustion of fossil fuels (coal,
56 natural gas, and oil) for energy and transportation, as well as for industrial processes and land-use
57 (IPCC 2014; Meehl et al., 2007; Sanderson et al., 2011). For the past ten years (2005 - 2014), the
58 average annual rate of increase has been 2.11 parts per million (ppm). An increase in the
59 atmospheric concentration of CO₂ and other greenhouse gases will lead to an increase of between
60 1.8 and 4°C in the global mean temperature (Meehl et al., 2007), and consequently these higher
61 CO₂ concentrations should be considered together with the increased temperatures. The number of
62 cold days and nights in the Northern Hemisphere has decreased globally/throughout the world, and,
63 from 1983 to 2012, were the warmest in the last 1,400 years (IPCC2013). Increases in CO₂ and
64 temperature induce complex effects on plant pathosystems.

65 Since both CO₂ and temperature are key variables that can affect plants and their diseases, climate
66 changes are influencing plant growth, plant diseases and, consequently, the global food supply
67 (Chakraborty and Newton, 2011).

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

73 Different approaches have been used to study the effect of increased temperature and CO₂ on
74 diseases, including laboratory and field studies, as well as modeling-based assessments (Salinari et

75 al., 2006). Phytotron-based studies permit a precise control of environmental parameters, such us
76 temperature, relative humidity, air, light, CO₂ concentration, air speed, leaf temperature and wetness
77 (Gullino et al., 2011). It is impossible to achieve such a degree of control under natural field
78 conditions.

79 Over the last decade, phytotrons have been used to study the effects of CO₂ enrichment and
80 temperature increases on infection rates for several pathosystems (Ainsworth and Long 2005;
81 Chakraborty 2005; Garrett et al. 2006; Grünzweig,2011; Pugliese et al. 2012 a, b; Ferrocino et al.
82 2013; Singh et al., 2014).

83 Foliar pathogens are influenced to a great extent by environmental conditions. Among the various
84 foliar diseases, downy mildew is expected to become more problematic due to the foreseen
85 temperature increases, which could directly or indirectly affect both the host and the pathogen
86 (Salinari et al., 2006). Warmer temperatures and a reduction in the length of humid weather periods
87 have reduced the severity of late blight potato disease (Schaap et al. 2011). On the other hand, , the
88 increase in severity and the earlier occurrence in *Phytophthora infestans* epidemics observed in
89 potatoes in Finland have been blamed on climatic warming and a lack of rotation (Hannukkala et
90 al., 2007). Basil downy mildew causes severe losses of basil, wherever this crop is grown, and
91 affects the production of this herb for its fresh consumption and pesto sauce production. The disease
92 was first reported in Italy in 2003 (Garibaldi et al., 2004) under high relative humidity (RH) and
93 warm temperature conditions, which are known to favor disease development (Garibaldi et al.,
94 2007).

95 In this study, the basil (*Ocimum basilicum*) - downy mildew (*Peronospora belbahrii*) pathosystem
96 was chosen to evaluate the effect of simulated elevated atmospheric CO₂ concentrations and
97 temperatures, as well as that of their interaction, under phytotron conditions. Six combinations of
98 CO₂ and temperatures were tested to establish their effect on disease development and on the
99 physiological parameters in order to observe the plants sensing and response to rising CO₂

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

102

103

104 **Material and methods**

105 Plant material

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

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

117

118 Artificial inoculation with *Peronospora belbahrii*

119

120 The inoculum was produced from one population of *P. belbahrii*, which was obtained from diseased
121 basil plants on a commercial farm in Piedmont (Northern Italy) and maintained on basil plants. A
122 suspension of viable sporangia of the pathogen was prepared by shaking the infected basil leaves in
123 100 ml of sterile water containing 2 µl of Tween 20; the suspension was adjusted with a

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

131

132 Experimental conditions

133 The effects of elevated CO_2 and temperature were studied in six physically and electronically
134 separated phytotrons, with 2 m wide x 2 m long x 2.5 m high internal dimensions (Gullino et al.,
135 2011). A 14/10-h day/night photoperiod was provided by two lighting systems (master-color CDM-
136 TD metallic iodure discharge lamps and TLD 18-830 Philips neon lamps. A gradual change in the
137 light intensity regime, resulting from three irradiance steps (0, 1/3, 2/3, 3/3) from 0 to $1200 \mu\text{mol m}^{-2}$
138 s^{-1} , was undertaken/introduced to simulate natural daylight. . Each phytotron was regulated in the
139 same way and maintained at high relative humidity, close to 85-95%.

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

142 The basil plants were maintained/kept in the phytotrons under six different combinations of
143 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
144 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
145 ppm CO_2 , 26-30 °C. In each trial, one environmental combination corresponded to one phytotron.
146 The phytotrons were randomized by changing the environmental conditions and combinations from
147 one trial to another. Three experimental trials were carried out as separate studies in 2014, under
148 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 $[\sum(n^{\circ} \text{ leaves} \times x \text{ 0-5}) / (\text{total leaves recorded})]$ 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

172 developed) of each basil plant, from the top, on ten randomly selected plants (one leaf/plant) at time
173 0 and at 8, 16 and 23 days (end of experiment) for the inoculated and non-inoculated basil plants.
174 Photosynthetic efficiency measurements were performed on five randomly selected leaves, using a
175 portable continuous-excitation type fluorimeter (Handy-PEA, Hansatech Instruments Ltd, Norfolk,
176 UK), according to the manufacturer's instructions, at time 0 and from day 8 every seven days, up to
177 the end of the trial for both the inoculated and non-inoculated plants.

178

179 Statistical analysis

180 All the analyses were conducted using the Superior Performing Software System SPSS 21.0 (SPSS
181 Inc., Chicago, IL, USA). Levene's Test was used to assess the homoscedasticity of Variance. Two-
182 way Anova was used to investigate the effect of each factor (CO₂ and temperature), and their
183 interactions, on disease incidence and severity caused by *P. belbahrii* on basil, and the means were
184 calculated according to Tukey's HSD test ($P < 0.05$). The average disease assessment values,
185 derived from counting, were arcsine-transformed before the statistical analysis was performed to
186 make the data closer to a normal distribution. The back-transformed mean values are shown in
187 Table 2.

188

189 **Results**

190

191 Effect on disease development

192 From a comparison of the trials, it has emerged that the assumptions of normality and
193 homoscedasticity were violated for disease incidence ($P < 0.05$), but were confirmed for disease
194 severity. The disease severity data were combined and analyzed as the average of three trials, using
195 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 CO₂ 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 CO₂), 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ conditions, a decreasing trend was observed particularly
213 pronounced and statistically significant at high CO₂ 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 CO₂
216 states, while only 850 ppm CO₂ was recorded 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 CO₂ conditions (Table 4), where
220 no influence on chlorophyll content was observed, compared with the non-inoculated basil plants.

221 Overall, the physiological measurements have confirmed that the disease development, which in
222 particular caused foliar damage at high CO₂ concentrations, led to a decrease in the physiological
223 performances.

224

225 **Discussion**

226 The connection between climatic changes and plant disease severity in several pathosystems has
227 received more and more attention (Pautasso et al., 2012; Pangga et al., 2013). Phytotrons have
228 frequently been used to simulate a climate change scenario, because they make it possible to
229 maintain total of the environmental conditions and thus to provide reproducible data, while
230 avoiding the high risk of fluctuations due to other factors that can be observed in natural
231 conditions. The present work was carried out in phytotrons in order to evaluate the effect of
232 increased carbon dioxide concentrations, and increased temperature, as well as the combination of
233 both these factors on basil downy mildew, in highly controlled environments. A good disease
234 level was reached in the three trials, thus making it possible to evaluate the effect of the different
235 quantities of CO₂ and temperature increases.

236 Two-way Anova has shown that the temperature and CO₂ of the six simulated environmental
237 conditions had a significant influence on the severity of basil downy mildew. A double
238 concentration of CO₂ caused a significant increase in downy mildew severity, in particular at
239 temperatures of 18-22 °C and 22-26°C. The effect of CO₂ on disease severity development was
240 observed at 26-30°C, although it was not always significant (Figure 1). Although elevated CO₂
241 alone had a significant effect on downy mildew severity, the increase in temperature also had a
242 significant effect on disease development. At average temperatures of 22 °C, which are favorable
243 for disease development (Garibaldi et al., 2007), elevated CO₂ may favor the growth of the
244 pathogen by increasing the sugar supply (Horsfall and Dimond, 1957; Stitt and Krapp, 1999;
245 Mahatma et al., 2009).

246 The direct effect of increased CO₂ values on plant diseases has been investigated less than the effect
247 of temperature increases. The effect of elevated CO₂ 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 CO₂. For example, von Tiedemann and Firsching (2000) found that
252 nitrogen-fixing legumes were more sensitive to elevated CO₂ and consequently to disease, while
253 elevated CO₂ did not affect the leaf rust disease of wheat. Grünzweig (2011) showed that high CO₂
254 could affect the susceptibility of *Onobrychis crista-galli* to powdery mildew. In other cases, it has
255 been shown that elevated CO₂ 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 the present
258 study, the impact of elevated CO₂ 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 CO₂ emissions, have accelerated
264 some of these changes. High CO₂ 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 CO₂ 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 CO₂ (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

272 assimilation rates (Nowak et al., 2004; Ainsworth and Long, 2005). However, as reviewed/pointed
273 out by Ainsworth and Rogers (2007), the maximum carboxylation rate and electron transport in C3
274 species are significantly reduced at elevated CO₂ and temperature conditions. This result is in
275 agreement with the photosynthetic efficiency (PI) (Table 3) measurements reported here both for
276 inoculated and non-inoculated basil plants. Moreover, the CO₂ sensing mechanism in guard cells is
277 still unknown and open to debate. Further free-air CO₂ enrichment (FACE) experiments are needed
278 to obtain more detailed knowledge on this key phenomenon (Ainsworth and Rogers, 2007). Similar
279 trends of PI were here observed in the leaf chlorophyll content, which is an indicator of
280 photosynthetic activity and chlorophyll stability for the conjugation of assimilates. SPAD
281 chlorophyll meters are frequently used for the quantitative measurement of foliar damage provoked
282 by different biotic and abiotic stresses (Bijanzadeh and Emam, 2010; Pugliese et al., 2010). The
283 measurement of the photosynthetic efficiency and the relative amount of chlorophyll in the leaf is a
284 rapid and effective way of establishing the healthy status of the photosynthetic machinery. A
285 significant decrease in the total chlorophyll content in susceptible genotypes of *Pennisetum*
286 *glaucum* affected by *Sclerospora graminicola* has been reported (Mahatma et al., 2009).
287 Considering the rise in CO₂ levels, it can be assumed that such changes could affect the severity of
288 basil downy mildew in the same way as that of other downy mildew pathogens (Salinari et al.,
289 2006). Carbon dioxide enrichment is another technique that is used to increase both yield and
290 profit (Huckstadt et al., 2013), but is not recommended for the cultivation of basil, due to the fact it
291 can intensify downy mildew and other diseases; as yet, this practice has not been applied in Italy.

292

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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 <i>P. belbahrii</i>	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 CO₂ 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	4	5	6
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					
		400-450 ppm			800-850 ppm			400-450 ppm			800-850 ppm			400-450 ppm			800-850 ppm		
Days of placing																			
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) Days of placing	Phytotron at 18-22 °C with CO2 at						Phytotron at 22–26°C with CO2 at						Phytotron at 26–30°C with CO2 at					
		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	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
11-17 days old plants 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 CO₂ 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)

