Research Papers

Effects of elevated CO₂ and temperature on interactions of zucchini and powdery mildew

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Summary. Effects of increased CO₂ and temperature on powdery mildew (Podosphaera xanthii) of zucchini (Cucurbita pepo), were evaluated under controlled conditions. Zucchini plants were grown in phytotrons under four different simulated climatic conditions: 450 ppm of CO₂ at standard (18°C night, 24°C day) and elevated temperatures (22°C night, 28°C day), elevated CO₂ (800 ppm) with standard temperature and elevated CO₂ (800 ppm) with elevated temperature (4°C higher than standard). Physiological responses of zucchini and pathogen development were studied. Under elevated CO₂ both healthy and infected zucchini plants grew better when temperature was lower. Elevated CO₂ generally caused no significant differences in pathogen development or disease severity, whereas elevated temperature stimulated the development of the pathogen. A combination of elevated CO₂ and temperature always stimulated the development of the pathogen and disease severity compared to standard conditions.

Key words: climate change, Cucurbita pepo, Podosphaera xanthii, epidemiology, phytotrons.

Introduction

Climate has changed in the recent past, due to increasing world population and economic activity, and it is predicted to change in the future (Smith et al., 2002). Atmospheric CO₂ concentration has increased from 367 ppm to 379 ppm in the period 2000-2006 (Le Treut et al., 2007), and is predicted to reach 730 to 1020 ppm by 2100 (Meehl et al., 2007). At the same time, an increase between 1.8 and 4°C in mean global temperature is expected, due to the rising concentration of CO₂ and other greenhouse gases (Meehl et al., 2007).

Since both CO₂ and temperature are key variables affecting plants and their diseases, potential influences of climate change on plant growth, global food supply and disease risk are attracting considerable research interest in many countries (Rosenzweig and Parry, 1994; Myneni et al., 1997; Harvell et al., 2002). Numerous studies have measured plant growth under conditions of elevated CO₂ and temperature. Despite the diversity of experimental approaches and study subjects, Morison and Lawlor (1999) concluded that increased CO₂ generally produced larger plants with more and/or larger organs, while warmer temperatures accelerated the rate of organ development and expansion but decreased organ life time. A meta-analysis, encompassing data from more than 120 peer-reviewed articles describing physiology and production in 12 large scale Free Air CO₂ Enrichment (FACE) experiments during the past 15 years, confirmed some results from previous chamber experiments, and also addressed general variances between species.

Increases in CO₂ and temperature are expected to induce complex effects on plant pathosystems. Although research on the effects of climate change
High CO₂ and temperature effects on powdery mildew

continues to be limited, new phytotron facilities are permitting study of effects of climate variables on infection rates in some pathosystems (Runion, 2003; Chakraborty, 2005; Garrett et al., 2006; Pugliese et al., 2010). An increase in production of defensive compounds and/or other changes in host physiology, morphology, or anatomy under elevated CO₂ could lead to reductions in disease incidence or severity for pathosystems such as Erysiphe graminis-barley and Colletotrichum gloeosporioides - Stylosanthes scabra (Runion et al., 1994; Hibberd et al., 1996; Chakraborty et al., 2000; Pangga et al., 2004). Several model approaches, such as climate matching and climatic mapping, have also been used to simulate and predict plant diseases under changing environments (Bourgeois et al., 2004). Increased downy mildew on grapevine in 2030, 2050 and 2080 was forecasted to occur in Acqui Terme, Italy, using a weather-disease combined model. Increased number of days during May and June with weather conditions favorable to downy mildew were predicted, due to the advance in the date of primary outbreaks (Salinari et al., 2007). An outcome of many studies and observations is increased powdery mildew severity on different hosts, as a result of temperature increases in mild climates (Boland et al., 2004).

The pathosystem zucchini (Cucurbita pepo) - powdery mildew (Podosphaera xanthii) was chosen to study the effects of elevated atmospheric CO₂ concentrations and temperatures and their interaction. Zucchini is a typical vegetable crop in the Mediterranean area. Powdery mildew, caused by the biotrophic fungus P. xanthii, is one of the most important diseases of this crop in many areas of the world, and yield losses due to the disease can reach 50% (Sherf and Macnab, 1986; Zitter et al., 1996). The disease is generally favoured by dry atmospheric conditions, moderate temperatures, reduced light intensity and succulent plant growth (Sitterly, 1978). Optimum temperature for spore germination is 28°C (Sitterly, 1978) and 20–27°C for disease development (Sherf and Macnab, 1986). Powdery mildew reduces yields by decreasing the size and/or number of zucchini fruit (McGrath and Thomas, 1996).

The present study was undertaken in phytotrons, where temperature and CO₂ concentration were manipulated to simulate possible future climate scenarios. Host physiological parameters (chlorophyll content, gas exchange activity and plant growth), and phytopathological factors, including changes in P. xanthii growth and infection structures, were evaluated.

Materials and methods

Growth of plants

Seeds of Cucurbita pepo cv. Genovese were sown in greenhouse (18–26°C, RH=70%, natural light). When the first seedling-leaves developed, about 10 days after sowing, plants were transplanted into pots (one plant/pot) containing 3:3:1 of a peat-clay-perlite substrate. Pots were then moved into three controlled environment phytotrons (PGC 9.2, TEC-NO.EL, Italy) (44 plants/phytotron), maintained at different concentrations of carbon dioxide (CO₂) and different temperatures, and at the relative humidity and photoperiod conditions outlined in Table 1.

### Table 1. Different environmental conditions used in phytotrons for assessing the effects of elevated CO₂ and temperature on interactions of zucchini and powdery mildew.

<table>
<thead>
<tr>
<th>Phytotron</th>
<th>Temperature (°C)</th>
<th>Carbon dioxide (ppm)</th>
<th>Relative Humidity (%)</th>
<th>PAR* (μmol m⁻² s⁻¹) (16 h photoperiod)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max (Day)</td>
<td>Min (Night)</td>
<td></td>
<td>Min (Day)</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>18</td>
<td>450</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>18</td>
<td>800</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>22</td>
<td>450</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>22</td>
<td>800</td>
<td>40</td>
</tr>
</tbody>
</table>

* PAR, Photosynthetically active radiation.
Concerning temperature, five trials were performed with controlled temperature (considered standard) of 18°C minimum during night to 24°C (maximum during day). Temperatures, air relative humidity and light changed gradually from day to night, to better simulate natural situations. The tested variables were: 450 ppm of CO₂ (standard) with standard temperature (experimental control), elevated CO₂ (800 ppm) with standard temperature, standard CO₂ with elevated temperature (4°C higher than standard) and elevated CO₂ with elevated temperature. Phytotron settings are summarized in Table 1.

The phytotrons used allowed environmental parameters (temperature, relative humidity, air CO₂ concentration, air speed, leaf temperature, leaf wetness) to be accurately controlled. Soil temperature and soil water content (absolute volumetric moisture content) were also monitored in the pot containers. Lighting was from two different sources to obtain the best spectrum for plant growth. Environmental parameters were also measured and recorded in order to fully characterize the internal phytotron environments.

Pathogen and inoculation procedure

*Podosphaera xanthii* inoculum was prepared from diseased plants maintained under greenhouse conditions. Conidia were collected from infected leaves, counted and adjusted using an haemocytometer (Bürker) in order to obtain conidial suspensions containing $5 \times 10^9$ – $1 \times 10^{10}$ conidia mL$^{-1}$. One drop of polysorbate 20 (Tween 20, Croda International Plc, Snaith, Goole, United Kingdom) was added to each suspension, and 2 mL was sprayed on the adaxial surface of each plant when the second true leaf was completely open (about 1 week after being moved into phytotrons).

At this time, plants to be inoculated were moved outside for a few minutes and sprayed with the inoculum. The plants were returned into the phytotrons. In each phytotron half of the plants were inoculated, while the others were left uninoculated as healthy experimental controls. During the experiments, inoculated and uninoculated plants inside each phytotron where not separated, to avoid influencing the microclimate in each phytotron unit. Assessments were carried out on primary infections and trials were terminated before the development of secondary infections.

### Assessment of the influence on host physiological activity and growth

The physiological activity of host plants was monitored through measurement of gas exchange and chlorophyll content index (CCI) in 2nd leaves (four replicates/leaf) on three infected plants and healthy plants in each phytotron. Gas exchange measurements were recorded, using a CO₂ and H₂O infra-red gas analyzer (ADC, Hoddesdon, UK) under open system. Intercellular concentration of CO₂ at 5 days after inoculation (dai), Ci (ppm), stomatal conductance at 5 dai, gs (mmol H₂O m⁻² s⁻¹), transpiration rate, E (mmol H₂O m⁻² s⁻¹) and assimilation A (μmol CO₂ m⁻² s⁻¹) at 3, 5 and 10 dai, were measured both for healthy and diseased plants grown under all conditions. Water use efficiency (WUE, μmol CO₂ mmol H₂O⁻¹) was calculated as ratio of assimilation (A) to transpiration rate (E). Chlorophyll content index (CCI) was determined at 10 dai, using a chlorophyll meter (SPAD-502, Minolta).

Growth of host plants was monitored by cutting and recording fresh and dry weights of shoots of three infected and three healthy plants from each environmental condition at 0, 10 and 15 dai. Dry weights were measured after shoots had been kept in a forced air oven for 48 h at 105°C. Number of open leaves, and number and maximum length of fruits at 15 dai were recorded and used as indexes of plant development. At least three plants were randomly sampled for measurement of each parameter at each sampling time.

Pathogen and disease assessments

Fungal development was assessed using microscopy, in order to evaluate: 1) maximum and minimum diameters of colonies measured at 3 dai, and then calculated as area (mm²), assuming each colony to be an ellipse; 2) number of hyphal tips (number of tips/colony) at 3 dai and number of conidiophores (number of conidiophores/colony) at 5 dai.

Three 1.5 cm diameter leaf disks, taken from the central part of the second leaves of three infected plants per phytotron at 3 and 5 dai, were detached and prepared for microscopy. The leaf disks were placed, adaxial surface up, into a Petri dish containing disks of absorbing paper soaked with a solution of ethanol-acetic acid (3:1). After complete chlorophyll extraction, usually about 20 h, leaf sections were moved to other Petri dishes containing...
absorbing paper soaked with water. After 1 h, the disks were moved to other Petri dishes containing
absorbing paper, soaked with lactoglycerol (lactic酸:glycerol:water, 1: 1: 1) for 24 h. Finally, leaf disks
were stained with 0.1% bromophenol blue and transferred to microscope slides for the assessment.

At 8 dai, for each environmental condition, six
individual powdery mildew colonies from the sec-
ond leaves of infected plants were collected and
transferred into a 2 mL Eppendorf tube containing
1 mL of water. Then the tube was placed on a vortex
shaker under 3400 rad s\(^{-1}\) for 3 min to obtain a well
mixed suspension. The concentration of conidia in
the suspension was determined with a microscope
slide haemocytometer (Bürker camera) and the aver-
age number of conidia produced by each colony was
calculated.

The number of powdery mildew spots or per-
centage of leaf surface infected was determined for
seven plants for each of the environmental condi-
tions, and translated into indices (0‒7) at 8, 10 and
15 dai, to show the disease incidence, according to
Horsfall and Cowling (1978). Translation standards
for these indices are shown in Table 2.

### Statistical analyses

Data were analyzed with analysis of variance
(ANOVA), using SPSS software for Windows (re-
lease 17.0; SPSS Inc., USA). Significance of differ-
ences between treatment means was evaluated using
the Tukey test (\(P\leq0.05\)).

### Results

#### Photosynthetic gas exchange

CCI was recorded at 10 dai and gas exchange ac-
tivity was monitored at 3, 5 and 10 dai in 2nd leaves
(Tables 3, 4 and 5). With 800 ppm CO\(_2\), CCI of healthy
plants was significantly decreased at higher temper-
atures (22 to 28°C). Average intercellular carbon di-
oxide (Ci) and stomatal conductance (gs) results of
three trials at 5 dai are shown in Table 3. Elevated
CO\(_2\) greatly enhanced intercellular carbon dioxide
(Ci) both in healthy and diseased plants. With higher
temperature the stimulation was even greater in
infected plants (Table 3). Stomatal conductance (gs)
was increased by elevated CO\(_2\) on healthy and in-
fected plants at 5 dai (Table 3). Considering only the
rise of temperature or CO\(_2\), CCI was significantly less
compared to standard conditions, while Ci was sig-
nificantly greater (Table 4).

Average assimilation (A) and transpiration rate
(E) results at 3, 5 and 10 dai in 5 trials are shown in
Table 5. Elevated CO\(_2\) greatly increased net photosyn-
thesis by accelerating assimilation (A), but not at
elevated temperatures (Table 5).

Transpiration rate (E) was not statistically differ-
ent (Table 5) for the different regimes. No differences
were observed in water use efficiency (WUE) among
the environmental regimes (Table 5).

The slope of the regression lines calculated for as-
similation and for the transpiration rates at 0, 3, 5 and
10 dai was always negative. Curves of regression
with determined coefficients are outlined in Table
6. Data from healthy and infected plants are mixed.
The analysis of variance showed that there were no
significant differences among temporal trends cal-
culated under the four different environmental re-
gimes (Table 6).

#### Plant growth and development

Plant shoot weights at 15 dai are outlined in Ta-
ble 7. No differences in fresh weights were detected
for the different environmental regimes. The warm-
er temperatures caused a decrease in dry weight,
especially on infected plants. Disease reduced plant
growth under all conditions, and the reduction was
highly significant under warmer temperature con-
ditions.

At standard temperatures, more fruits were pro-
duced by healthy plants under elevated CO\(_2\) than at
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Table 3. Mean chlorophyll content indices (CCI), intercellular carbon dioxide (Ci) and stomatal conductance (gs) in 2nd leaves of zucchini plants grown in different temperature and CO₂ regimes. Data are shown as means ± SE (n ≥5). Average results of five trials.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>CO₂ (ppm)</th>
<th>CCI, 10 dai</th>
<th>Ci (ppm), 10 dai</th>
<th>Gs (mmol m⁻² s⁻¹), 10 dai</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>healthy</td>
<td>infected</td>
<td>healthy</td>
</tr>
<tr>
<td>18‒24</td>
<td>450</td>
<td>23.7±1.27 a</td>
<td>20.3±1.06 a</td>
<td>365.8±22.04 b</td>
</tr>
<tr>
<td>18‒24</td>
<td>800</td>
<td>25.5±1.30 a</td>
<td>20.2±1.62 a</td>
<td>594.6±32.43 a</td>
</tr>
<tr>
<td>22‒28</td>
<td>450</td>
<td>24.9±1.53 a</td>
<td>12.9±1.89 b</td>
<td>353.7±7.88 b</td>
</tr>
<tr>
<td>22‒28</td>
<td>800</td>
<td>19.4±1.55 b</td>
<td>9.7±1.74 b</td>
<td>595.4±8.14 a</td>
</tr>
</tbody>
</table>

* Means of each parameter accompanied by the same letter are not significantly different at P=0.05 (Tukey’s test).

Table 4. Mean chlorophyll content indices (CCI) and intercellular carbon dioxide (Ci) in 2nd leaves of zucchini plants grown in different temperature and CO₂ regimes considering temperature or CO₂. Data are shown as means ± SE (n ≥5). Average results of five trials.

<table>
<thead>
<tr>
<th>Parameter value</th>
<th>CCI</th>
<th>Ci (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ (ppm)</td>
<td>450</td>
<td>18.3±0.82 a</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>15.7±0.90 b</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>18‒24</td>
<td>21.7±0.67 a</td>
</tr>
<tr>
<td></td>
<td>22‒28</td>
<td>12.8±0.86 b</td>
</tr>
</tbody>
</table>

*Means of each parameter accompanied by the same letter are not significantly different at P=0.05 (Tukey’s test).

450 ppm CO₂: on the contrary, higher temperature reduced fruit development (Table 7). At 800 ppm of CO₂ and under higher temperatures, the plants had more fully opened leaves (Table 7), although the leaves were smaller than at lower temperature.

Development of powdery mildew and disease index

Results of microscopy observations are shown in Table 8.

Colony area, number of hyphal tips, conidiophores and conidia per colony were significantly greater at temperature ranging from 22 to 28°C compared to standard conditions. Disease indices (0‒7) also varied in different trials due to the quantity of inoculum, but were never influenced only by increased CO₂. On the contrary, elevated temperature and CO₂ significantly stimulated disease index (Table 8).

There was a clear increment of growth of the pathogen, fecundity and severity of the disease observed at the higher temperature-higher CO₂ combination compared with control temperature combinations, in particular with higher CO₂ at standard temperatures.

Table 5. Mean assimilation (A), transpiration rate (E), and water use efficiency (WUE) in 2nd leaves of zucchini plants grown in different temperature and CO₂ regimes. Data are shown as means of three trials.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>CO₂ (ppm)</th>
<th>A+ (μmol CO₂ m⁻² s⁻¹)</th>
<th>E (mmol m⁻² s⁻¹)</th>
<th>WUE (μmol CO₂/mmol H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18‒24</td>
<td>450</td>
<td>20.52±2.47 a</td>
<td>4.93±0.87 a</td>
<td>4.2±1.15 a</td>
</tr>
<tr>
<td>18‒24</td>
<td>800</td>
<td>30.72±6.76 b</td>
<td>4.46±0.54 a</td>
<td>6.9±1.33 a</td>
</tr>
<tr>
<td>22‒28</td>
<td>450</td>
<td>17.49±3.52 a</td>
<td>3.84±0.39 a</td>
<td>4.6±1.26 a</td>
</tr>
<tr>
<td>22‒28</td>
<td>800</td>
<td>22.66±2.61 a</td>
<td>4.13±0.68 a</td>
<td>5.5±0.69 a</td>
</tr>
</tbody>
</table>

*Means of each parameter accompanied by the same letter are not significantly different at P=0.05 (Tukey’s test).
In our study, both healthy and powdery mildew-infected zucchini plants grew better under elevated CO₂. Similar results have been previously reported for several host-pathogen combinations (Wechsung et al., 1995; Usuda and Shimogawara, 1998; Morison and Lawlor, 1999; Wand et al., 1999; Kaddour and Fuller, 2004; Ainsworth and Long, 2005; Pugliese et al., 2010).

With elevated CO₂ and temperature, the vegetative-reproductive balance of zucchini plants was in

**Table 6.** Linear regression analyses of assimilation (A) and transpiration rate (E) for zucchini plants grown in different temperature and CO₂ regimes.

<table>
<thead>
<tr>
<th>Phytotron</th>
<th>A+ (μmol CO₂ m⁻² s⁻¹)</th>
<th>Regression curve</th>
<th>R²</th>
<th>E (mmol m⁻² s⁻¹)</th>
<th>Regression curve</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (18‒24 °C, 450 ppm CO₂)</td>
<td>-0.73 a</td>
<td>y = -0.73 x + 1.94</td>
<td>0.867</td>
<td>-0.44 a</td>
<td>y = -0.44 x + 3.56</td>
<td>0.801</td>
</tr>
<tr>
<td>2 (18‒24 °C, 800 ppm CO₂)</td>
<td>-1.71 a</td>
<td>y = -1.71 x + 8.22</td>
<td>0.825</td>
<td>-0.37 a</td>
<td>y = -0.37 x + 0.68</td>
<td>0.793</td>
</tr>
<tr>
<td>3 (22‒28 °C, 450 ppm CO₂)</td>
<td>-1.02 a</td>
<td>y = -1.02 x + 3.86</td>
<td>0.874</td>
<td>-0.31 a</td>
<td>y = -0.31 x + 4.62</td>
<td>0.849</td>
</tr>
<tr>
<td>4 (22‒28 °C, 800 ppm CO₂)</td>
<td>-1.95 a</td>
<td>y = -1.95 x + 1.92</td>
<td>0.893</td>
<td>-0.28 a</td>
<td>y = -0.28 x + 3.31</td>
<td>0.906</td>
</tr>
</tbody>
</table>

**Table 7.** Mean fresh and dry weights of plant shoots, numbers and maximum lengths of fruits, and numbers of open leaves at 15 days after inoculation, for zucchini plants grown in different temperature and CO₂ regimes. Data are shown as means ± SE (n ≥5).

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>CO₂ (ppm)</th>
<th>Shoot weight (g)</th>
<th>Zucchini fruits</th>
<th>Open leaves (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fresh</td>
<td>Dry</td>
<td>Number</td>
</tr>
<tr>
<td>18‒24</td>
<td>450</td>
<td>115±16.86 a</td>
<td>82.0±2.71 a</td>
<td>9.3±0.783 ab</td>
</tr>
<tr>
<td>18‒24</td>
<td>800</td>
<td>111±11.34 a</td>
<td>85.6±2.81 a</td>
<td>11.8±1.582 a</td>
</tr>
<tr>
<td>22‒28</td>
<td>450</td>
<td>101±5.36 a</td>
<td>78.1±4.32 a</td>
<td>9.4±0.852 b</td>
</tr>
<tr>
<td>22‒28</td>
<td>800</td>
<td>116±13.67 a</td>
<td>75.9±6.76 a</td>
<td>8.8±0.314 b</td>
</tr>
</tbody>
</table>

*Means of each parameter accompanied by the same letter are not significantly different at P=0.05 (Tukey’s test).

**Table 8.** Mean pathogen parameters and disease indices for powdery mildew on zucchini plants grown in different temperature and CO₂ regimes. Data are shown as means ± SE (n ≥5).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>CO₂ (ppm)</th>
<th>Colony area (mm²)</th>
<th>Hyphal tips (number/colony)</th>
<th>Conidiophores (number/colony)</th>
<th>Spores/colony (number)</th>
<th>Disease Index (0–7)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>18‒24</td>
<td>450</td>
<td>0.35±0.033 a</td>
<td>10.3±0.83 a</td>
<td>0.4±0.35 a</td>
<td>5315±840 a</td>
<td>2.8±0.33 a</td>
</tr>
<tr>
<td>18‒24</td>
<td>800</td>
<td>0.31±0.031 a</td>
<td>9.5±0.71 a</td>
<td>2.0±0.89 a</td>
<td>4199±645 a</td>
<td>2.9±0.35 a</td>
</tr>
<tr>
<td>22‒28</td>
<td>450</td>
<td>0.52±0.058 b</td>
<td>13.4±0.69 b</td>
<td>4.1±0.37 b</td>
<td>8491±684 b</td>
<td>3.4±0.41 ab</td>
</tr>
<tr>
<td>22‒28</td>
<td>800</td>
<td>0.68±0.133 b</td>
<td>17.3±1.81 c</td>
<td>11.4±2.27 c</td>
<td>9871±721 b</td>
<td>4.0±0.36 b</td>
</tr>
</tbody>
</table>

*Means of each parameter accompanied by the same letter are not significantly different at P=0.05 (Tukey’s test).

* The disease index refers to combined assessments at 8, 10 and 15 dai.

**Discussion**

In our study, both healthy and powdery mildew-infected zucchini plants grew better under elevated CO₂. Similar results have been previously reported for several host-pathogen combinations (Wechsung et al., 1995; Usuda and Shimogawara, 1998; Morison and Lawlor, 1999; Wand et al., 1999; Kaddour and Fuller, 2004; Ainsworth and Long, 2005; Pugliese et al., 2010).
favour of vegetative development: plants produced more leaves and less fruit, especially in diseased plants. The same plants had lower dry weights and similar fresh weights to plants grown at standard temperature and CO₂ concentration, which corresponded to greater water content and, so, to greater susceptibility to pathogens.

In short, elevated CO₂ reduced stomatal conductance (gs), stimulated intercellular concentration of CO₂ (Ci), and assimilation (A). With higher temperature, the results were similar, except that there was a reduction of CCI and smaller increase of assimilation. The reduction of chlorophyll was caused by more rapid leaf senescence under higher temperature. Intercellular concentration of CO₂ in plants grown at elevated carbon dioxide was significantly increased, even compared to values observed in plants grown under elevated temperature. Under elevated CO₂ and temperature, plants developed much more rapidly and warmer temperature accelerated the rate of organ development and expansion but reduced the duration (Morison and Lawlor, 1999), but elevated CO₂ did not completely compensate for this negative effect. So, under elevated CO₂ and temperature, healthy zucchini plants aged rapidly with less chlorophyll in 2nd leaves at 10 dai, and produced more but smaller leaves, fewer and smaller fruits, resulting in lower dry weight at the end of the trials (15 dai).

Considering the assimilation data, zucchini plants probably belong to the group of plant which are able to cope well with excess carbohydrate, similarly to trees, which show large sink capacities (Davey et al., 2006; Ainsworth and Rogers, 2007).

At standard temperatures, elevated CO₂ alone did not affect growth of P. xanthii; on the contrary, in combination with an increase of temperature pathogen development was always stimulated. These results suggest that the increase of CO₂ per se had no effect on disease development, while temperatures per se significantly increased pathogen development. When temperatures are favorable to powdery mildew, elevated CO₂ may favour growth of these pathogens by increasing sugar supply for plant growth (Hibberd et al., 1996). Similar to the results of Jenkyn and Bainbridge (1978), infection of powdery mildew increased plant respiration and reduced chlorophyll content index and stomatal conductance, thus reducing the net photosynthesis and plant weight under all environmental conditions, though not always significantly. Increased respiration due to more rapid pathogen development under higher temperature may explain why infected plants had higher Ci than those grown under the standard environmental condition.

Four different sets of environmental conditions were tested in the present study: 450 ppm of CO₂ with standard and elevated temperatures; 800 ppm of CO₂ with standard and elevated temperatures. Florides and Christodoulides (2009) reported that an increase in the CO₂ level is directly correlated to an increase in temperatures, so our purpose was to consider a possible future scenario in which temperatures and CO₂ will both be greater. Our results showed that under the tested conditions, zucchini powdery mildew development is more influenced by the combination of CO₂ air concentration and temperature than by CO₂ level alone. However, increased CO₂ can induce some changes in host physiology and anatomy, for instance linked to the observed increased assimilation under high concentration of CO₂, that can be involved in the activation of plant defense mechanisms.

Considering that the rising concentrations of CO₂ and other greenhouse gases will lead to an increase in global temperature, we can assume that the increasing levels of CO₂ are likely to indirectly affect powdery mildew of zucchini, and may also similarly affect other powdery mildew pathogens.

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**Literature cited**


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