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RESEARCH PAPERS

Evaluating severity of leaf spot of lettuce, caused by *Allophoma tropica*, under a climate change scenario

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Summary. Climate changes, particularly increases in temperature and CO_2, are seriously challenging agriculture, and are one of the main factors that should be considered in the emergence of new diseases and their potential spread. Six trials were carried out to evaluate the effects of increased temperature and CO_2 on the severity of leaf spot of lettuce, caused by *Allophoma tropica* (syn. *Phoma tropica*), a pathogen that was first observed on lettuce in northern Italy in 2011. Temperature, CO_2 and their interactions were significant factors ($P<0.0001$) influencing incidence and severity of leaf spot on lettuce. Temperatures between 22 and 26°C were the most favourable to the pathogen, and increased disease incidence and severity. Reductions in disease incidence and severity were observed at lower (18–22°C) and higher (26–30°C) temperatures. Concentrations of CO_2 ranging from 800 to 850 ppm increased disease incidence and severity at all the temperature ranges tested, and these effects were greatest at 22-26°C. Analysis of these results could be useful for mid-term agricultural planning at a regional scale, so that crops and their varieties can be adapted to anticipated future climate trends.

Key words: phytotron, leafy vegetable, CO_2, temperature, *Lactuca sativa*, Phoma leaf spot.

Introduction

Climate changes, and increases in temperature and CO_2 in particular, are seriously challenging agriculture and affecting pathosystems, together with other global change components, such as air, water and soil pollution, the long-distance introduction of exotic host and pest/pathogen species and globalization of markets (Pautasso *et al.*, 2012). Southern Europe is expected to be seriously affected by these climate changes, with consequent extensification of its cropping systems and abandonment of some crops and cultivation areas (Bindi and Olesen, 2011). Climate changes have recently been indicated as one of the main factors that should be taken assessed when considering the emergence of new diseases and their potential spread (Garibaldi and Gullino, 2010; Garibaldi *et al.*, 2014).

Such a perspective has stimulated the search for solutions, through different approaches, to mitigate the negative effects of climate changes. Phytotrons have proved to be useful tools to simulate climate change scenarios, as the most important environmental parameters, i.e. temperature and atmospheric CO_2 concentrations, can be varied, and the effect of such changes on plants and their pathogens can be evaluated. Although the first research on effects of climate changes on plant pathogens was concentrated on field crops, such as cereals and grapevine (Coakley *et al.*, 1999; von Tiedemann and Firsching 2000; Garrett *et al.*, 2006; Salinari *et al.*, 2006; White *et al.*, 2006; Bregaglio *et al.*, 2013), more recent research has evaluated climate change effects on foliar and soil-borne pathogens of vegetable and ornamental

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crops (Pugliese *et al*., 2010, 2012a, 2012b; Ferrocino *et al*., 2013; Chitarra *et al*., 2015; Gilardi *et al*., 2016a, 2016b).

Many Mediterranean countries, including Italy, have long been areas appropriate for production of leafy vegetables, including lettuce (*Lactuca sativa* L.), in greenhouses and in open fields. Lettuce is a popular crop throughout the world, and its consumption has been increasing as a consequence of the attention being paid to healthy eating habits and to the availability of convenience foods. Lettuce is also a crop that has been frequently studied to evaluate the effects of climate changes, and because it is susceptible to several pathogens (Mortensen 1985; Wheeler *et al*., 1993; Ferrocino *et al*., 2013). Lettuce is susceptible to many fungal diseases, caused by soilborne and foliar pathogens (Davis *et al*., 1997; Blancard and Maisonneuve 2003). Recently, many new diseases have been reported on lettuce, most of which have been linked to the intensification of the production systems, to the use of new varieties corresponding with new consumer preferences (i.e. ready-to-eat salads), and to the quick and simultaneous spread of some pathogens through the use of infested/infected seeds in globalized markets (Gullino *et al*., 2014).

The present study was undertaken to evaluate the effects of increased temperature and atmospheric CO_2 concentrations, which were simulated by working in phytotron conditions, on the severity of the leaf spot of lettuce, caused by *Allophoma tropica* (syn. *Phoma tropica*). This pathogen was first observed on lettuce in northern Italy in 2011 (Garibaldi *et al*., 2012), and was renamed by Chen *et al.* (2015).

Material and methods

Experimental layout

Six trials were carried out in 2016 at the Center for Innovation in the Agro-environmental Sector (AGROINNOVA), at the University of Torino, in Grugliasco (Italy), in six physically and electronically separated phytotrons (each with internal dimensions of 2 m wide × 2 m long × 2.5 m high). These were operated with a 14 h light/10 h dark photoperiod, provided by two lighting systems (mastercolour CDM-TD metallic iodine discharge lamps and TLD 18-830 Philips neon lamps) (Gullino *et al*., 2011). Eight environmental combinations were tested under completely controlled conditions. The following temperature and CO_2 concentration combinations were tested in the first set of trials: 1) 400–450 ppm CO_2, 14–18°C; 2) 800–850 ppm CO_2, 14–18°C; 3) 400–450 ppm CO_2, 18–22°C; 4) 800–850 ppm CO_2, 18–22°C; 5) 400–450 ppm CO_2, 22–26°C, and 6) 800–850 ppm CO_2, 22–26 °C.

In the second set of trials (Table 1), since it had been observed that leaf spot caused by *A. tropica* was

Table 1. Treatments and experimental details for six trials carried out on *Allophoma tropica* on lettuce (cv. Elisa).

	First set of trials			**Second set of trials**		
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**
CO_2 × Temperature	400-450 ppm CO_2, 14-18 °C 800-850 ppm CO_2, 14-18 °C 400-450 ppm CO_2, 18-22 °C 800-850 ppm CO_2, 18-22 °C 400-450 ppm CO_2, 22-26 °C 800-850 ppm CO_2, 22-26 °C			400-450 ppm CO_2, 18-22 °C 800-850 ppm CO_2, 18-22 °C 400-450 ppm CO_2, 22-26 °C 800-850 ppm CO_2, 22-26 °C 400-450 ppm CO_2, 26-30 °C 800-850 ppm CO_2, 26-30 °C		
Sowing date	22/03/16	12/04/16	29/04/16	11/08/2016	26/09/16	10/10/16
Transfer of plants to phytotrons	1/04/16	3/05/16	20/05/16	01/09/16	7/10/16	31/10/16
Inoculations with *Allophoma tropica*	11/04/16	11/05/16	27/05/16	8/09/16	19/10/16	08/11/16
First symptoms	13/04/16	16/05/16	31/05/16	12/09/16	21/10/16	14/11/16
Final disease assessment and end of the trial	18/04/16	19/05/16	3/06/16	16/09/16	26/10/16	17/11/16

significantly reduced at the lowest temperature, the temperature range of 14–18°C was replaced with a higher temperature range, 26–30 °C, which was combined with CO_2 concentration at a standard 400–450 ppm and double concentration, 800–850 ppm.

A total of five to six pots (one pot = one experimental unit per phytotron per pathosystem per trial) were examined.

The environmental parameters (light, temperature, humidity and atmospheric CO_2) inside the phytotrons were continuously monitored and kept constant. The light intensity regime, which resulted from three irradiance steps (0, 1/3, 2/3, 3/3) from 0 to 1,200 μmol m^{-2} s^{-1}, and relative humidity (RH), were regulated in the same way in all the phytotrons. The phytotrons were randomized by changing the environmental conditions and combinations during the first and second set of trials (Table 1).

Plant material and artificial inoculation

Lettuce plants (cv. Elisa; T&T) were grown in 2 L capacity plastic pots, filled with a steamed (90°C for 30 min) white pea:perlite (80:20 v:v) mix (Turco Silvestro). At least 20 plants were present in each pot. The plants were kept in a nursery compartment in a glasshouse at 22–23°C before being transferred to the phytotrons.

The PHT30 coded strain of *Allophoma tropica,* isolated from infected plants, was used for the inoculation of the lettuce plants. The pathogen was grown on potato dextrose agar (PDA; Merck) amended with streptomycin sulphate, for 7–10 d at 20–23 °C, with a 12 h photoperiod. Suspensions containing 5×10^5 conidia mL^{-1} of the pathogen were used to inoculate 20 to 25-d-old plants. The inoculum suspensions were applied 7 d after the plants had been transferred to the phytotrons. After inoculation, the pots were placed under a plastic support (100 × 100 × 50 cm) and covered with a transparent polyethylene film (50 mm thick) for 24h, to maintain relative humidity at 95–100%.

Disease assessments and statistical analysis

The inoculated plants were observed for disease development. Disease incidence (DI, expressed as percent of infected leaves) was evaluated on 50 leaves/treatment, by counting the number of affected leaves 7–8 d after inoculation. Disease severity (DS, expressed as percent of affected leaf area) was evaluated using a rating scale of 0 to 5 (0 = no symptoms; 1 = up to 5% of leaf area affected; 2 = 6 to 10% affected; 3 = 11 to 25% affected; 4 = 26 to 50% affected; and 5 = 51 to 100 % of leaf area affected). DS was calculated using the formula:

$$DS = [\sum(n^\circ \text{ leaves} \times x_{0-5}) / (\text{total number of leaves recorded})]$$

where $x_{0-5} = (x_0=0;\ x_1= 5\%;\ x_2=10\%;\ x_3=25\%;\ x_4=50\%$ or $x_5=75\%)$.

The data were analysed by using univariate ANOVA, with Tukey's HSD test, with SPSS software 22.0. Statistical analysis of the results was carried out using the Levene test to check for homogeneity of variance. Two-way ANOVA was used to investigate the effects of temperatures and CO_2 concentrations, as well as the CO_2 × temperature interaction, on DI and DS. Standard errors of means are presented as bars in the text figures presented below.

Results

The inoculation method used in these trials led to high disease incidence and severity in all the trials, with consistent results in both sets of trials. The first leaf spot symptoms were observed 3–6 d after inoculation with the pathogen.

DS data from the first and second sets of the trials were combined and analysed using ANOVA two-way analysis of variance, with SPSS software 22, because 'trial' did not significantly influence lettuce leaf spot in the first set (DI P=0.061; DS P=0.199) and second set of the trials (DI P=0.78; DS P=0.094).

Two-way analysis of variance confirmed that the temperature, CO_2 and their interactions were statistically significant factors (P<0.0001) of influence on disease incidence and severity of *A. phoma* on lettuce.

Temperatures between 22 and 26°C are the most favourable for the pathogen, and caused significantly more disease (incidence and severity) than the other two temperature regimes. Significant reductions in disease incidence and severity were observed at lower (18–22°C) and higher temperatures (26–30°C) (Figures 1 and 3). High atmospheric CO_2 concentrations significantly increased disease incidence and severity for all three temperature regimes. This effect was amplified for DS at 22–26°C, the most favourable for disease development (Figure 2).

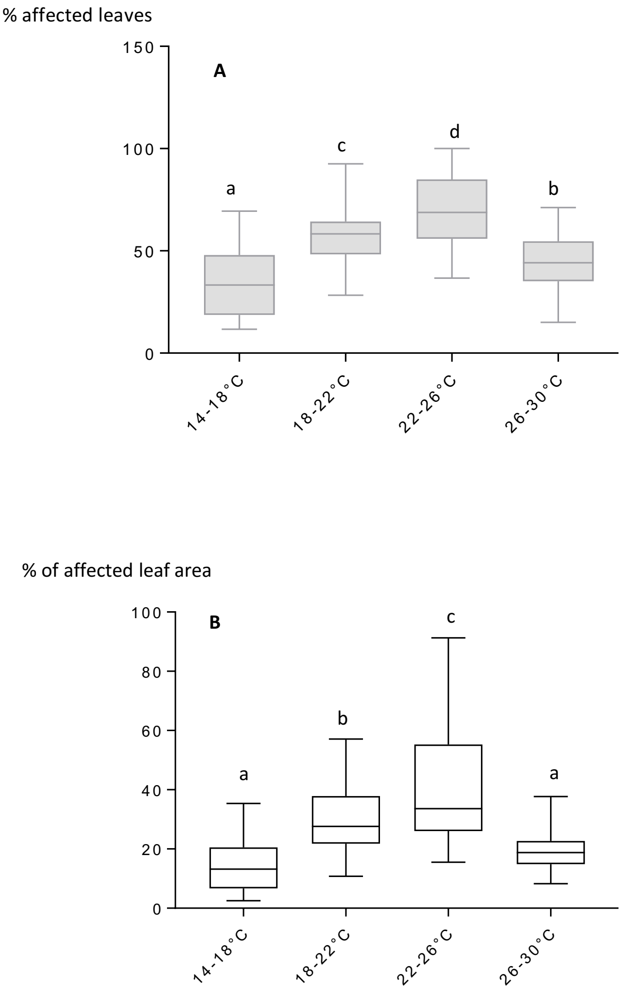

Figure 1. Effects of different temperature regimes (14–18, 18–22, 22–26 and 26–30°C) on the disease caused by *Allophoma tropica* on lettuce (cv. Elisa). A) Mean disease incidence, % of affected leaves. B) disease severity, % of leaf are affected. Mean value of trials 1–6. Data accompanied by the same letter are not significantly different, according to Tukey's Test (*P*<0.05). The boxes represent the interquartile range (IQR) between the first and third quartiles, and the lines inside the boxes represent the median (2nd quartile). The whiskers denote the least and the greatest values within 1.56 IQR from, respectively, the first and third quartiles.

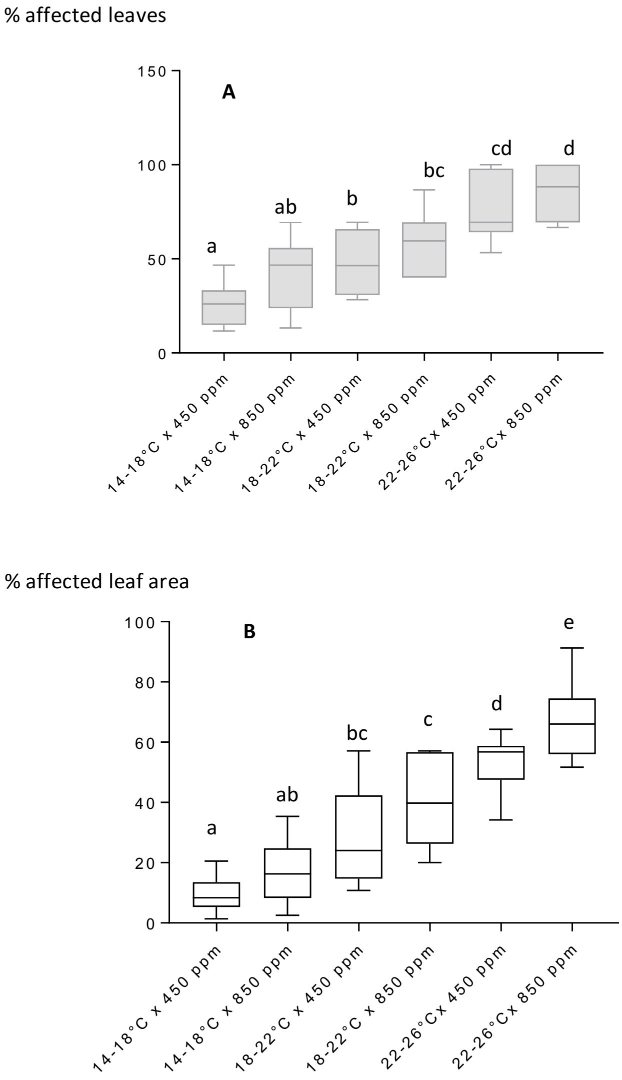

Figure 2. Effects of different CO_2 and temperature (14–18, 18–22 and 22–26°C) combinations on leaf spot, caused by *Allophoma tropica* on lettuce (cv. Elisa). A) Mean disease incidence, % of affected leaves. B) Mean disease severity, % of affected leaf area. Mean values for trials 1–3. Data accompanied by the same letter are not significantly different, according to Tukey's Test (*P*<0.05). The boxes represent the interquartile ranges (IQR) between the first and third quartiles, and the lines inside the boxes represent the median (2nd quartile). The whiskers denote the least and greatest values within 1.56 IQR, respectively, from the first and third quartiles.

Discussion

The pathogen *A. tropica*, with temperate temperature requirements, is ideal for studies to evaluate effects of climate changes on plant diseases. Recent epidemiological studies have shown that this pathogen is more aggressive at temperatures of 20 and 25°C, which are typical under tunnel house conditions in northern Italy during spring. At these temperatures,

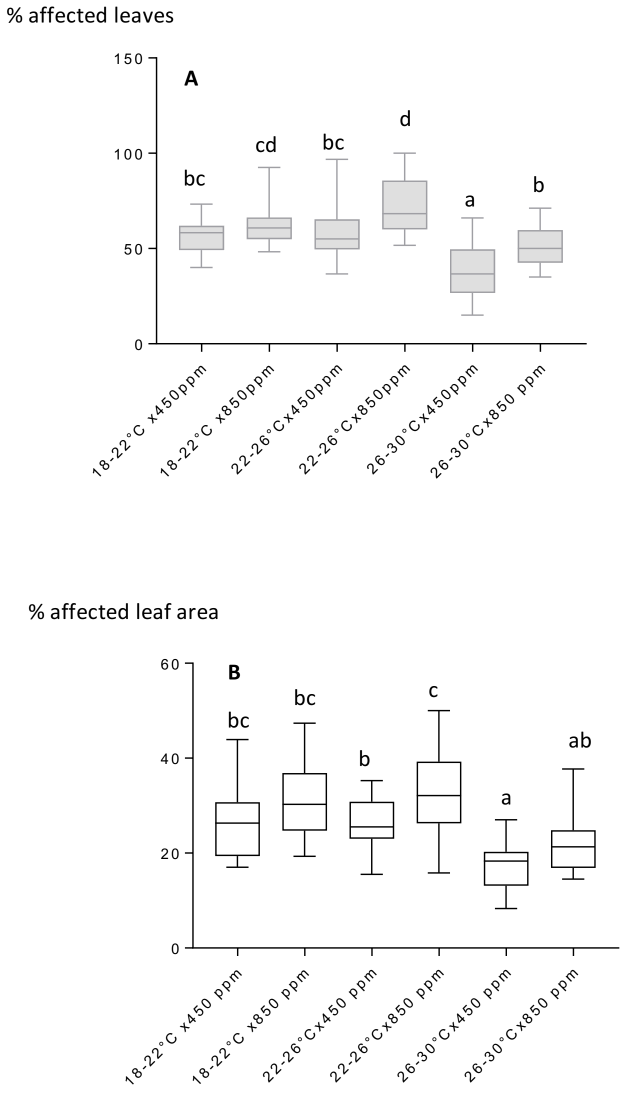

Figure 3. Effects of different CO_2 and temperature (18–22, 22–26 and 26–30°C) combinations on leaf spot caused by *Allophoma tropica* on lettuce (cv. Elisa). A) Mean disease incidence, % of affected leaves. B) disease severity, % of leaf area affected. Mean values for trials 4–6. Data accompanied by the same letter are not significantly different, according to Tukey's Test ($P<0.05$). The boxes represent the interquartile ranges (IQR) between the first and third quartiles, and the lines inside the boxes represent the median (2nd quartile). The whiskers denote the least and the greatest values within 1.56 IQR from, respectively, the first and third quartiles.

short periods of high relative humidity (1–6 h) are sufficient to cause significant crop losses (Gilardi *et al*., 2017).

Studies carried out to understand effects of climate changes on pathogens, have shown that effects of increased temperatures and CO_2 concentrations variy according to the pathosystem. In some cases, increased temperature is the most important factor, while in others increased CO_2 is more relevant. In still other cases, increased temperature and CO_2 interact to affect disease severity. As underlined by Chakraborty (2013), to improve confidence in future predictions, a broad range of scenarios and case studies need to be evaluated. Empirical research, using factorial combinations of CO_2 and temperature, is useful to develop future prediction models, since the current models are not based on findings from multifactorial studies. A study carried out in phytotrons with the for the lettuce/*Fusarium oxysporum* f. sp. *lactucae* pathosystem showed that severity of Fusarium wilt increased with elevated temperature, while elevated CO_2 did not affect severity of this disease (Ferrocino *et al*., 2013).

In the present study, which was carried out under controlled conditions in phytotrons in short duration experiments, the elevated temperatures alone did not result in increased disease incidence or severity, caused by *A. tropica* on lettuce. Instead, there was clear effect of elevated atmospheric CO_2 on leaf spot of lettuce for the 22 to 26°C temperature regime.

Analysis of these results could be useful for midterm agricultural planning at a regional scale, to adapt crops and their varieties to anticipated future climate trends. This is particularly important for agricultural systems in the Mediterranean and South European regions, which are likely to be more vulnerable to climate changes than other European regions (Bindi and Olesen, 2011).

Increases in temperature and CO_2 concentrations are among the main factors that should be assessed when considering the emergence of new diseases and their potential spread. Here we have demonstrated that temperature, CO_2 concentrations and their interactions were significant factors influencing on disease incidence and severity for *A. phoma* leaf spot on lettuce. Temperatures of 22 to 26°C were the most favourable to the pathogen, and CO_2 cpncentrations of 800 to 850 ppm increased disease incidence and severity at this temperature range in comparison to the other climate regimes assessed.

These results provide new information on the effect of elevated temperature and CO_2 concentrations for a new disease of lettuce, highlighting the presence of different responses to climate change in different pathosystems. More research in this field is needed, at a regional scale, to provide plant breeders with greater insights supporting development of crop varieties that could adapt to future weather conditions, and which could show resistance to pathogens likely to become predominant in the future, as a result of forecasted climate changes.

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