Maria Lodovica Gullino, Giovanna Gilardi, Angelo Garibaldi, EFFECT OF A CLIMATE CHANGE SCENARIO ON *FUSARIUM EQUISETI* LEAF SPOT ON WILD ROCKET AND RADISH UNDER PHYTOTRON SIMULATION,

Phytoparasitica, 45 (3), 2017, pagg. 293-298, DOI 10.1007/s12600-017-0590-1

EFFECT OF A CLIMATE CHANGE SCENARIO ON *FUSARIUM EQUISETI* LEAF SPOT ON WILD ROCKET AND RADISH UNDER PHYTOTRON SIMULATION

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Abstract This study was undertaken by simulating the effects of increasing the temperature and CO_2 values on the incidence and severity of *F. equiseti* on wild rocket (*Diplotaxis tenuifolia*) and radish (*Raphanus sativus*), under phytotron conditions. Two sets of 3 trials were carried out in which eight different temperature and CO_2 combinations were tested: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; 7) 400-450 ppm CO_2, 14–18 °C; 8) 800-850 ppm CO_2, 14–18 °C. The temperature and CO_2 levels were significant factors of influence on disease incidence (DI) and severity (DS) in all the trials, and their combination significantly influenced the DI and DS of *F. equiseti* leaf spot on both hosts. Disease incidence and severity increased on wild rocket at 850 ppm of CO_2, in comparison to 450 ppm, in each tested temperature range. The highest CO_2 value on radish, for all the tested temperature regimes, caused an increase in DI and DS, which resulted statistically significant at the highest tested temperature range. The results obtained in this study add more concern to the possible negative effects of the spread of *F. equiseti* on vegetables in Italy as well as in other areas suffering from increased temperatures as a consequence of climate changes.

Keywords: *Raphanus sativus, Diplotaxis tenuifolia,* increased temperature and CO_2, vegetable crops

INTRODUCTION

Fusarium equiseti is a soil inhabitant that commonly occurs in tropical and subtropical regions (Booth 1978; Bosch and Mirocha 1992). It is generally considered a weak pathogen that can infect the seeds, roots, tubers and fruit of several crops, such as asparagus, cotton, cowpeas, cumin, ginseng, lentils, pine, potato and sugar beet, on which it causes a variety of symptoms (Farr and Rossman 2016).

Some vegetables that are grown intensively in Italy for ready-to-eat salad mixes and fresh consumption have recently been found to be new hosts of this species: wild rocket (*Diplotaxis tenuifolia*) (Garibaldi et al. 2015), cultivated rocket (*Eruca sativa*) (Garibaldi et al. 2011), lettuce (*Lactuca sativa*) (Garibaldi et al. 2016b) and radish (Garibaldi et al. 2017). Most of the new hosts are grown intensively as monocultures or in succession, both of which favours the survival of the pathogen, which is a good saprotroph, in soil or crop debris. The sudden appearance of the pathogen in northern Italy could be related, at least partially, to the increase in temperature detected in

northern Italy as a consequence of climate changes. In fact, recent studies have shown that the *F. equiseti* strains isolated in Italy have high temperature requirements. Lettuce is very susceptible to *F. equiseti*, in particular at 25 and 30°C; at such temperatures, 1 and 3 hours of leaf wetness have been found to be sufficient to cause high disease incidence and severity. At least 12 hours of leaf are instead necessary to cause high losses at lower temperatures (Garibaldi et al. 2016a). The pathogen is also favoured on wild rocket by temperatures of 30 and 35°C; under such conditions, only one hour of leaf wetness is sufficient to cause significant levels of the disease, while longer periods (6 and 12 hours) of high relative humidity are necessary to cause significant losses at lower temperatures (Garibaldi et al. 2016a). The use of wild and cultivated rocket as a salad leaf has increased in Italy with about 3,600 ha (Casati and Baldi 2016), while radish is generally used in rotation with other vegetables grown under greenhouse or in open field, for a total of 1,323 ha (923 ha in open field and 400 ha under protection) (ISTAT 2015).

Since there are indications that suggest a possible effect of climate change on the recent spread of this pathogen on new hosts, which are economically important in the Mediterranean region, a study was undertaken in which the effects of increasing temperature and CO_2 values on the incidence and severity of *F. equiseti* on wild rocket (*Diplotaxis tenuifolia*) and radish (*Raphanus sativus*) were simulated under phytotron conditions.

MATERIALS AND METHODS

Experimental layout A total of six experimental trials (3 on rocket and 3 on radish) were carried out in 2016 at the Center of Competence Agroinnova, in Grugliasco, Italy. Six physically and electronically separated phytotrons (2 m wide x 2 m long x 2.5 m high internal dimensions) with a 14/10-h day/night photoperiod, provided by two lighting systems (master-color CDM-TD metallic iodine discharge lamps and TLD 18-830 Philips neon lamps), were used (Gullino et al. 2011). Eight environmental combinations were tested under completely controlled environmental conditions. A total of five-six pots (one pot = one experimental unit / phytotron per trial) were examined for both pathosystems.

The phytotrons were randomized by changing the environmental conditions and combinations during the first and second sets of trials (Table 1).

Plant material and experimental conditions Cultivar Grazia wild rocket (*Diplotaxis tenuifolia*), (Enza Zaden, Italy) was grown in 2 L vol. plastic pots filled with a steamed (90°C for 30 minutes) white peat : perlite mix, 80:20 v/v (Turco Silvestro, Albenga, Italy). At least 20 plants were present

in each pot. cv. Flamboyant radish plants (Sgaravatti, Italy) were grown in 2L plastic pots filled with the same substrate (15 plants/pot). The wild rocket and radish plants were kept in a glasshouse at 22-23°C before being transferred to the phytotrons.

Two sets of trials were carried out (Table 1). The wild rocket and radish plants were kept in the phytotrons under eight different temperature and CO_2 combinations: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; 7) 400-450 ppm CO_2, 14–18 °C; 8) 800-850 ppm CO_2, 14–18°C.

The environmental parameters (light, temperature, humidity and CO_2) inside the phytotrons were monitored continuously and kept constant. The light intensity regime, resulting from three irradiance steps (0, 1/3, 2/3, 3/3) from 0 to 1200 μmol m^{-2} $s^{-}1$, and relative humidity (RH) were regulated in the same way in all the phytotrons. Relative humidity values close to 85-95% were maintained in each phytotron during the trials.

Artificial inoculation of the pathogen The strains coded as Feq 5/14 of *Fusarium equiseti* from wild rocket and Feq 14/14 of *Fusarium equiseti* from radish were used for the inoculation of the two tested hosts. Both strains were grown on potato dextrose agar (PDA, Merck, Darmstadt, Germany), amended with streptomycin sulphate for 7-10 days at 20-23 °C, with a 12 hour photoperiod. A suspension containing a concentration of $1x10^7$ conidia/ml of the pathogen was sprayed onto twenty to twenty-five-day-old plants, 7 days after their transfer to the phytotrons. After the artificial inoculation, the pots were placed under a plastic support (100×100×50 cm) and covered with a transparent polyethylene film (50 μm thick) for 24h, in order to keep the relative humidity at 85-90%.

Disease assessment and statistical analysis The inoculated plants were checked for disease development, and the number of infected leaves was counted on 50 wild rocket and 20 radish leaves, respectively per treatment, 7 days after inoculation. Disease incidence (DI) was expressed as the percent of infected leaves. Disease severity (DS) was recorded by adopting a scale from 0 to 5 (0 = no symptom; 1 = up to 5 % infected leaf area; 2 = 6 to 10% infected leaf area; 3 = 11 to 25% infected leaf area; 4 = 26 to 50% infected leaf area; 5= 51 to 100 % infected leaf area). Disease severity was calculated by using the formula: $DS= [\sum(n° \text{ leaves} \times x_{0-5}) / (\text{total of leaves recorded})]$ with $x_{0-5} = (x_0=0; x_1= 5\%; x_2=10\%; x_3=25\%; x_4=50\%; x_5=75\%)$.

The data were analysed by means of univariate ANOVA with Tukey's HSD test using SPSS software 22.0. A statistical analysis of the results was carried out using the Levene test to check for the homogeneity of variance. One-way ANOVA was used to investigate the effect of the increasing temperatures and CO_2 and CO_2 × temperature on disease incidence (DI, expressed as % of infected leaves) and disease severity (DS, expressed as % of affected leaf area). The standard errors are marked with error bars in the figures.

RESULTS

The data from the disease assessments of the six trials were combined and analyzed using the ANOVA one-way analysis of variance, after the homogeneicity of variances had been verified for DS (P>0.05), because 'trial' had been found not to be a significant factor of influence on *Fusarium equiseti* incidence (p=0.09) and severity (p=0.07) in any of the six trials (Table 1). The same analysis confirmed that the temperature (p<0.001) and CO_2 levels (p<0.001) were significant factors of influence on disease incidence (DI) and severity (DS) in trials 1-6. The Tukey HSD post-hoc test showed that the combination of the CO_2 and temperature factors significantly influenced the incidence (p =0.0009) and severity of *F. equiseti* leaf spot on both hosts (Figures 2 and 4).

In the case of wild rocket, the DI and DS were significantly higher at temperatures from 18-22°C to 26-30°C than at 14 to 18°C (Figure 1). Disease incidence and severity increased at 850 ppm of CO_2, in comparison to 450 ppm, or each tested temperature range. Such an increase was statistically significant for disease incidence at 14-18 °C and for disease severity at 26-30 °C (Figure 2).

Temperature was a significant factor on disease incidence and severity caused by *F. equiseti* on radish (Figure 3). The artificial inoculation with *F. equiseti* on this host also caused more severe symptoms, in terms of DI and DS, at the highest tested temperature regimes of 18-22, 22-26°C and 26-30°C, with significantly lower attacks at 14-18 °C (Figure 3).

The CO_2 concentration also significantly influenced *Fusarium equiseti* leaf spot severity (p<0.0001) in the six trials carried out on radish. The Tukey HSD post- hoc test confirmed that the combined CO_2 and temperature factors significantly influenced DI and DS (p <0.0001) of *F. equiseti* (Figure 4). At all the temperature regimes tested, the highest CO_2 value caused an increase in DI and DS, which resulted statistically significant at the highest tested temperature range (Figure 4).

DI was also significantly higher in the case of radish at 14-18°C and 26-30°C at 850 ppm of CO_2 than at 450 ppm (Figure 4), while DS resulted significantly affected by the increase in CO_2 at 26-30°C (Figure 4).

DISCUSSION

The experiments carried out in phytotrons, by simulating a climate change scenario, with increased temperature and CO_2 values, showed the highest virulence on rocket and radish of *F. equiseti* at the highest tested temperatures, thus providing further support to the hypothesis that the recent spread of this pathogen in northern Italy on some new hosts is linked to climate changes (Garibaldi et al. 2016 a). Colombo et al. (2007) studying the temperature and precipitation variations in Italy, refer an increase of tropical nights with T_{min} >20 °C and at the same time a decrease of frost days T_{min} < 0 °C starting from the end of 1970s. Moreover, the trend of temperature and precipitation for the period 1991–2000 showed a still significant increase of winter temperatures over Italy starting from 1980. Furthermore, based on the Italian Meteorological Service Network, 2015 resulted the warmest year in northern Italy with an average increase of temperature of +1.5 ° C, with the month of July has become the hottest since 1800.

The present study provided the evidence of the effect of climate changes on *F. equiseti* causing a disease incidence and severity generally higher, on both of the tested crops, in the presence of the highest tested CO_2 level and temperatures. The fact that the combination of high temperature and high CO_2 levels significantly influences disease incidence and severity makes *F. equiseti* a serious threat to many crops, due the broad range of hosts of this pathogen. Some features of this pathogen increase the risk posed by its appearance: in the case of rocket, the pathogen was found to be a seed contaminant (Gilardi et al. 2017), and this explains the rapid appearance of this pathogen on new hosts in different geographical areas. The capability of *F. equiseti* to produce mycotoxins, in particular nivalenol, diacetoxyscirpenol and zearalenone, potentially increases the risk posed by its spread (Bottalico 1988; Bosch 1992; Jimenez et al. 1997; Vujanovic et al. 2006; Goswami et al. 2008; Stepien et al. 2013). The effects of increased temperature and CO_2 values on mycotoxin production on some leafy vegetables is currently under investigation. Moreover, atmospheric CO_2 enrichment has shown to be able of influences a wide range of plant processes. Grünzweig (2011) showed as global climate change, such as elevated CO_2, potentially impact the population fitness and alter plant communities of the Mediterranean legume *Onobrychis crista-galli* with an effects on the performance of progeny and on their susceptibility to powdery mildew. The phenotypic

adaptation to environmental variation due to maternal effects could be of high significance for evolutionary consequences.

The results obtained in this study add more concern to the possible negative effects of the spread of *F. equiseti* on vegetables in Italy, as well as in other areas affected by increased temperatures as a consequence of climate changes.

F. equiseti represents a good example of a fungus that in the past was considered a weak pathogen (Rai 1979; Reuveni, 1982; Elmer 1996; Punja 1997; Chimbekujwo 2000; Kosiak et al. 2005; Punja et al. 2008), but which has become more serious and invasive, and which represents a serious threat to new hosts. In fact, climate changes can have an important effect on the phytosanitary situation of many hosts, and can lead to the quick spread of the pathogen to crops grown in succession, in part due to its possible seed transmission.

Acknowledgements This project has received funding from the EU Horizon 2020 research and innovation programme under grant agreement No. 633999 (EMPHASIS). The authors would like to thank Federico Berta and Andrea China Gallo for the technical support, and Marguerite Jones for the language revision.

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Table 1 Main information on the six trials carried out on the *Fusarium equiseti*-wild rocket and *Fusarium equiseti*-radish pathosystem

	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			400-450 ppm CO_2, 18–22 °C		
	800-850 ppm CO_2, 14-18 °C			800-850 ppm CO_2, 18–22 °C		
	400-450 ppm CO_2, 18–22 °C			400-450 ppm CO_2, 22–26 °C		
	800-850 ppm CO_2, 18–22 °C			800-850 ppm CO_2, 22-26 °C		
	400-450 ppm CO_2, 22–26 °C			400-450 ppm CO_2, 26-30 °C		
	800-850 ppm CO_2, 22-26 °C			800-850 ppm CO_2, 26-30 °C		
Sowing date of wild rocket and radish	22/03/16	22/04/16	11/05/16	5/08/16	26/09/16	21/10/16
Transfer to the phytotrons of rocket and radish plants	1/04/16	3/05/16	20/05/16	16/06/16	7/10/16	31/10/16
Inoculations with *Fusarium equiseti*	11/04/16	11/05/16	27/05/16	23/08/16	19/10/16	8/11/16
Final disease assessment and end of the trial	18/04/16	19/05/16	3/06/16	17/09/16	29/10/16	21/11/16

Figure 1 Effect of three temperature regimes on the incidence (DI, expressed as % of affected leaves) and severity (DS, expressed as % of affected leaf area) of *Fusarium equiseti* on wild rocket (cv. Grazia). Mean value of trials 1-6. Columns superscripted with the same letter are not significantly different for DI and DS, respectively at $p \leq 0.05$ (Tukey's Test)

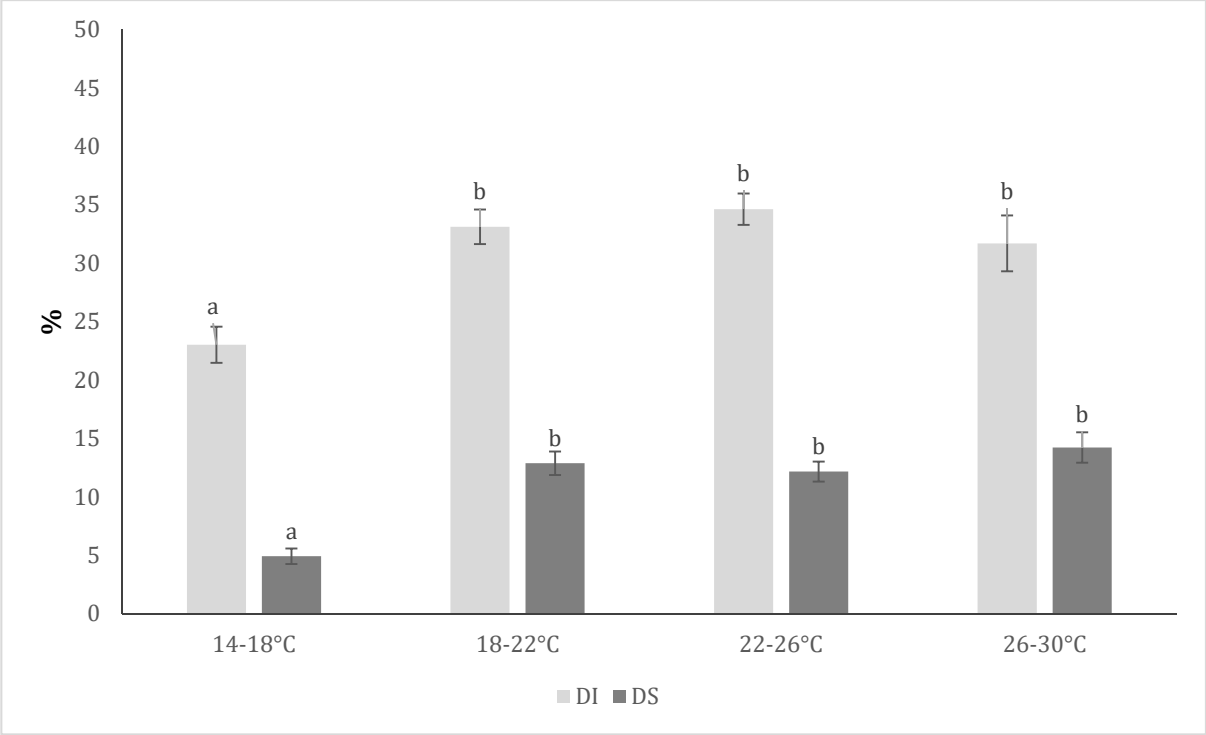

Figure 2 Effect of different CO_2 and temperature combinations on the incidence (DI, expressed as % of affected leaves) and severity (DS, expressed as % of affected leaf area) of *Fusarium equiseti* on wild rocket (cv.Grazia). Mean value of trials 1-6. Columns superscripted with the same letter are not significantly different for DI and DS, respectively at $p \leq 0.05$ (Tukey's Test)

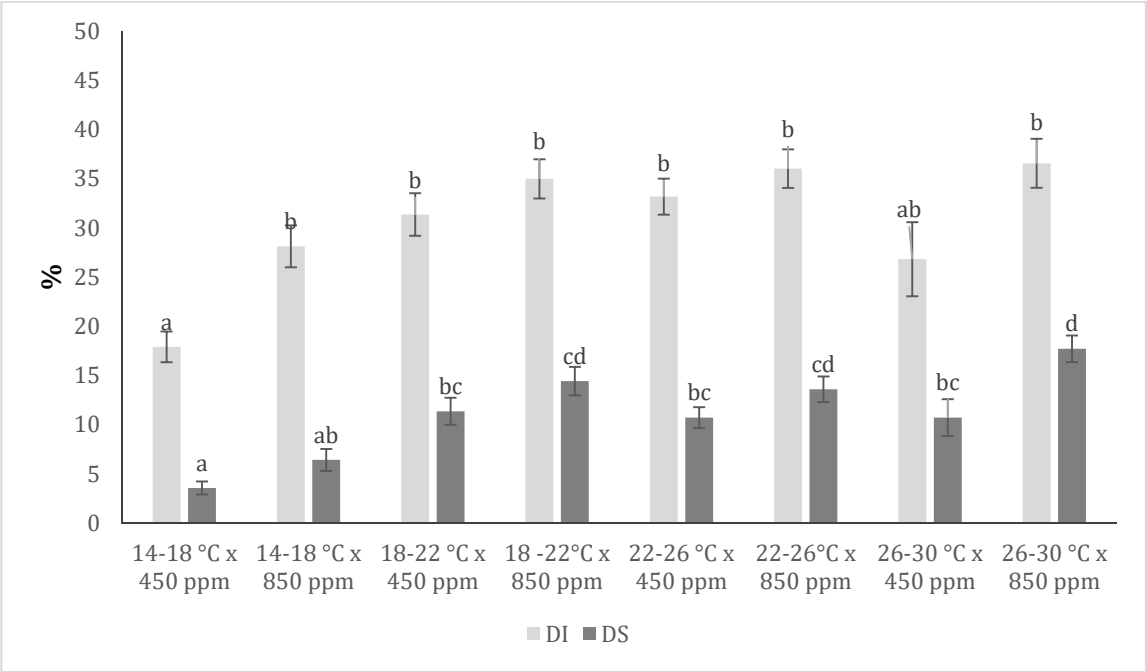

Figure 3 Effect of three temperature regimes on the incidence (DI, expressed as % of affected leaves) and severity (DS, expressed as % of affected leaf area) of *Fusarium equiseti* on radish (cv. Flamboyant). Mean value of trials 1-6. Columns superscripted with the same letter are not significantly different for DI and DS, respectively at $p \leq 0.05$ (Tukey's Test)

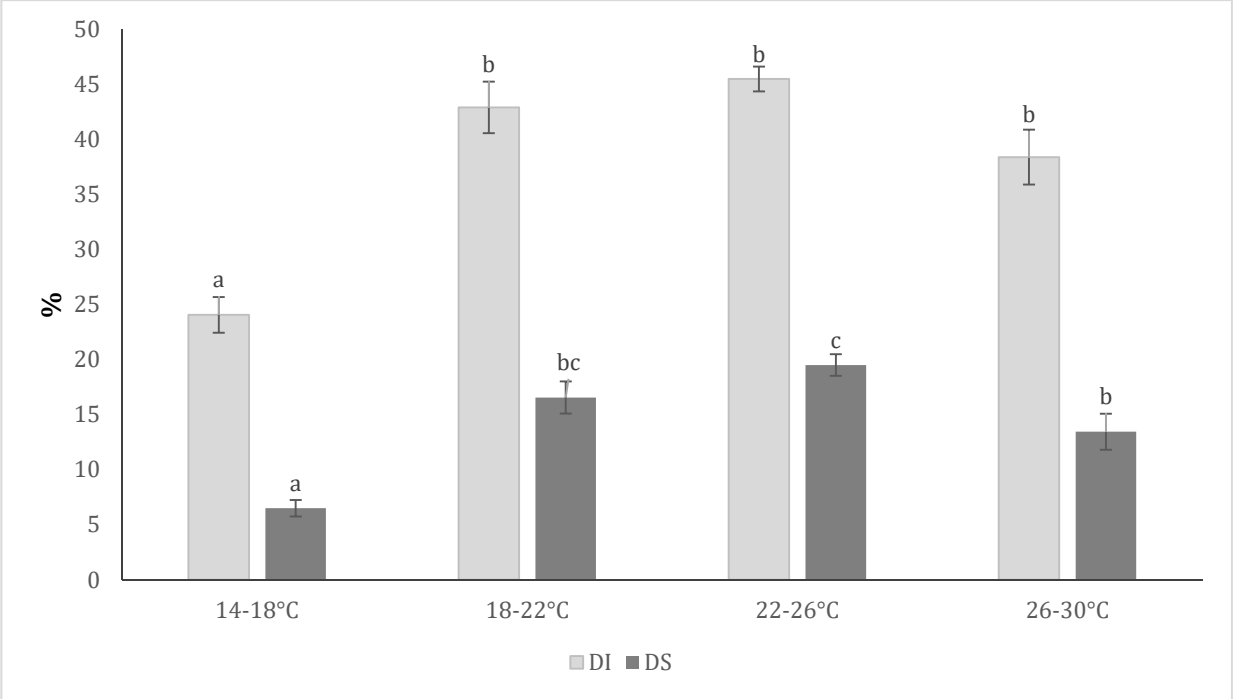

Figure 4 Effect of different CO_2 and temperature combinations on the incidence (DI, expressed as % of affected leaves) and severity (DS, expressed as % of affected leaf area) of *Fusarium equiseti* on radish (cv. Flamboyant). Mean value of trials 1-6. Columns superscripted with the same letter are not significantly different for DI and DS, respectively at $p \leq 0.05$ (Tukey's Test)