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Simulated elevated atmospheric CO_2 and temperature affect the severity of bean and pelargonium rust

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Abstract Experimental trials were carried out on bean *(Phaseolus vulgaris* L.)/*Uromyces appendiculatus* F. Strauss and pelargonium [*Pelargonium zonale* (L.) L'Hér.ex Aiton]/ *Puccinia pelargonii-zonalis* Doidge pathosystems, under phytotron conditions, to evaluate the effects of simulated elevated atmospheric CO_2 concentrations, ranging from 800 to 850 ppm, compared with standard CO_2, ranging from 400 to 450 ppm, and temperatures, ranging from 14 to 18, 18 to 22, 22 to 26 and 26 to 30 °C. A total of eight CO_2 and temperature combinations were tested to establish their effects on the development of bean and pelargonium rusts. A doubled concentration of CO_2, at the same temperature regime, was found to lead to an increase in *U. appendiculatus* disease severity on the beans. The combined CO_2 and temperature factors significantly influenced the severity ($p = 0.049$) of the rust caused by *U. appendiculatus*. Moreover, *Puccinia pelargonii–zonalis* was observed to be more severe on pelargonium at the lowest tested temperatures, that is, between 14 and 22 °C. A high CO_2 regime was shown to significantly increase disease severity at such temperatures. At the highest tested temperatures, that is, between 26 and 30 °C, which are generally not favourable for rust development, the increase in CO_2 had no significant effect on disease severity.

Keywords Climate changes · *Phaseolus vulgaris, Pelargonium zonale* · *Uromyces appendiculatus* · *Puccinia pelargonii-zonalis*

Introduction

Several researches have been carried out over the past few years to evaluate the effects of increases in atmospheric CO_2 and temperature, which have been associated with global climate changes, on plants and their diseases (Coakley et al. 1999; Ainsworth and Long 2005; Chakraborty et al. 2002; 2013; Ghini et al. 2008; Gautam et al. 2013; Pugliese et al. 2012a, b; Ferrocino et al. 2013; Gilardi et al. 2016). Phytotrons can be used to simulate future climate change scenarios because they permit environmental parameters, such as temperature, relative humidity, light, CO_2 concentration, air speed, leaf temperature and wetness to be manipulated (Gullino et al. 2011), in order to evaluate the behaviour of plants and their pathogens. Plant pathogens, particularly foliar pathogens, are affected in various ways in different pathosystems by increased CO_2 and temperature (Ainsworth and Long 2005; Mina and Sinha 2008; Grünzweig 2011; Pugliese et al. 2010, 2012a, b; Chakraborty 2013).

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The effects of global environmental changes on host-pathogen interactions are complicated, and are still not well known. Moreover, they show a great deal of tolerance to environmental conditions, and this tolerance is expected to decline under the predicted climate change scenarios, although it could remain unchanged or even become more prevalent (Helpher 2014). Several rusts are more dependent on humidity during the initial infection, and shifts in humidity levels caused by climate change will probably affect their development more than expected (Dixon et al. 2010). Previous research focused on foliar pathogens, such as rust, and analysed the results of climate variations on winter wheat stripe rust (Coakley 1979). Increased CO_2 may encourage the production of a high plant biomass and, consequently, a high concentration of carbohydrates in the host tissue may promote the development of biotrophic fungi, such as rusts (Chakraborty et al. 2002).

The influence of temperature also causes different effects on cereal rust isolates. Stripe rust (*Puccinia striiformis* Westend) can adapt to and benefit from high temperatures, becoming more aggressive (Mboup et al. 2012). The disease resistance to *P. recondita* Rob ex.Desm. of different wheat cultivars has also been reported to show temperature sensitivity, which could not simply be attributable to gene expression resistance (Gerecheter-Amitai et al. 1984; Gregory et al. 2009). Thus, predisposed crops to more severe infections, particularly in the absence of resistant varieties (Kaur et al. 2008; Gregory et al. 2009; Gautam et al. 2013).

The relationships between environmental conditions and rust diseases have been studied for different pathosystems. The relative humidity and air temperatures are important factors that are conductive to the infection and progress of rust. The optimum air temperatures for the germination of wheat rust urediniospores generally ranges from 12 to 15 °C, with the process stopping at temperatures >35 °C (De Vallavieille-Pope et al. 1995). Tall fescue (*Festuca arundinacea* Schreb.) stem rust and perennial ryegrass (*Lolium perenne* L.) *Puccinia graminis* subsp. *graminicola* Pers.:Pers. stem rust do not survive at temperatures below 13 °C, and their incidence may increase because of global warming (Pfender and Vollmer 1999). Similarly, *Phakopsora pachyrhizi* Syd. P. Syd., soya bean rust, may be positively affected by warmer winters in the USA, thus offering the pathogen an earlier start (Park et al. 2008). The optimum air temperatures for the germination of bean rust urediniospores ranges from 17 to 27 °C (Groth

and Mogen 1978), while the optimum air temperature for the infection of *Puccinia pelargonii-zonalis* on pelargonium was shown to be 16 °C, and to stop at 32 °C (Harvood and Raabe 1979).

The impact of combined environmental factors, such as temperature and CO_2, on bean and geranium rust infections is basically unknown.

The aim of this study was to evaluate the effect of increased CO_2 and temperature, and their interactions, on two pathosystems: *Phaseolus vulgaris*/*Uromyces appendiculatus* and *Pelargonium zonale* / *Puccinia pelargonii-zonalis,* under phytotron conditions.

Materials and methods

Plant material

Bean seeds belonging to the cv. Edna (Seminis Vegetable Seeds) were sown in 2 L vol plastic pots filled with a steamed (90 °C for 30 min) white peat : perlite mix, 80:20 v/v (Turco Silvestro, Albenga, Italy). At least five bean plants were present in each pot. The plants were kept at 22–23 °C, until the second true leaf phenological stage, before being transferred to the phytotrons.

Pelargonium plants of cv. Pac® Bergpalais ℗, obtained from a local nursery, were grown in 3.5 L plastic pots filled with the same substrate.

Experimental conditions

Trials were carried out in 2014 and 2015, under six physically and electronically separated phytotrons (2 m wide × 2 m long × 2.5 m high), with a 14/10-h day/night photoperiod provided by two lighting systems (master-color CDM-TD metallic iodure discharge lamps and TLD 18–830 Philips neon lamps). A gradual change in the light intensity regime, which resulted from three irradiance steps (0, 1/3, 2/3, 3/3) from 0 to 1200 μmol m^{-2} s^{-1}, was introduced to simulate natural daylight (Gullino et al. 2011). Light and RH were regulated in the same way in all the phytotrons. A high relative humidity (85–95 %), was maintained in each phytotron during the trials. The environmental parameters (light, temperature, humidity and CO_2) inside the phytotrons were monitored continuously and kept constant (Gullino et al. 2011).

The bean and pelargonium plants were kept in the phytotrons under eight different temperature and CO_2

ranges and combinations. In a first set of trials (Figs. 1 and 3), the following combinations were considered: 1) 400 to 450 ppm CO_2, 18 to 22 °C; 2) 800 to 850 ppm CO_2, 18 to 22 °C; 3) 400 to 450 ppm CO_2, 22 to 26 °C, 4) 800 to 850 ppm CO_2, 22 to 26 °C, 5) 400 to 450 ppm CO_2, 26 to 30 °C; 6) 800 to 850 ppm CO_2, 26 to 30 °C. In a second set of trials (Figs. 2 and 4), because it had been observed that the bean and pelargonium rust severity was significantly reduced at the highest temperature, instead of the temperature range from 26 to 30 °C, a lower temperature range from 14 to 18 °C, was combined with CO_2 at a standard (400 to 450 ppm CO_2) and double concentration (800 to 850 ppm) in the following combinations: 7) 14 to 18 °C; 800 to 850 ppm CO_2; 8) 14 to 18 °C, 400 to 450 ppm CO_2. The experiments were repeated three times for each pathosystem, under the eight environmental combinations, and under completely controlled environmental conditions.

A total of six pot replicates/experiment were used for both pathosystems.

The phytotrons were randomized by changing the environmental conditions and combinations during the first and second sets of experiments.

Artificial inoculation

One population each of *U. appendiculatus* and *P. pelargonii-zonalis,* collected from diseased plants from two commercial farms in Piedmont (Northern Italy), were multiplied on bean and pelargonium plants, respectively, and used for inoculation. The urediniospore suspension from both pathogens was prepared by shaking bean or pelargonium infected leaves in 100 ml of sterile water containing 2 μl of Tween 20; the suspension was adjusted to $1–5 \times 10^4$ urediniospores ml^{-1} for both pathogens. The spores were applied to the bean and pelargonium plants, using a hand-held sprayer (10 ml capacity), 7–10 days after their transfer to the phytotrons, by spraying 1 ml/pot on about 15–20 leaves/ pot. After artificial inoculation, the pots were placed under a plastic support ($100 \times 100 \times 50$ cm) and covered with a transparent polyethylene film (50 μm thick) for 7 days in order to keep the relative humidity at 85–90 %.

Pathogen measurement and statistical analysis

The inoculated plants were checked for disease development, and the number of infected leaves was counted, starting from the appearance of the first symptoms. Rust severity was evaluated on both hosts by counting the number of uredia on 15 and 20 leaves from each bean and pelargonium pot, only considering the leaves present when artificial inoculation was carried out.

All data were subjected to a Levene test to check for the homogeneity of variance. Disease severity data from the first and second sets of the bean and pelargonium trials were combined and analysed using the ANOVA one-way analysis of variance with SPSS software 22, because 'trial' had not been found to be a significant factor of influence on bean rust severity ($p = 0.612$) in the first and second ($p = 0.74$) set of trials. The means were spread according to Tukey's HSD test ($p < 0.05$).

Results

One-way analysis of variance confirmed that the temperature ($p = 0.003$), CO_2 levels ($p = 0.001$) and their interactions were significant factors ($p = 0.05$) of influence on disease severity. The Tukey HSD post-hoc test showed that the combination of the CO_2 and temperature factors significantly influenced bean rust severity ($p = 0.049$) (Fig. 1). Bean rust did not develop at the highest temperatures (26 to 30 °C) for either of the tested CO_2 regimes. The same analysis, conducted in the second set of trials (Figs. 2 and 5), confirmed that the CO_2 concentration significantly influenced bean rust severity ($p = 0.005$). In the presence of standard CO_2 values, bean rust was more severe at the lowest tested temperatures (18 to 22 and 14 to 18 °C), while disease severity, expressed as the number of uredia/leaf, was significantly lower at high temperatures (Fig. 1). The high CO_2 regime in general caused a significant increase in disease severity at the temperatures ranging from 14 to 26 °C (Figs. 2 and 5).

All the considered factors (CO_2 and temperature) as well as their combinations, significantly influenced the rust severity ($p < 0.0001$) caused by *P. pelargonii– zonalis* (Figs. 3, 4 and 5). The pelargonium rust was also more severe on pelargonium at the lowest tested temperatures, that is, from 14 to 22 °C (Figs. 3, 4 and 5). At such temperatures, a high CO_2 regime significantly increased disease severity. At 22 to 26 °C, in the presence of very low disease severity, the high CO_2 regime caused a slight increase in the number of uredia/leaf.

When the temperatures ranged between 26 and 30 °C, disease severity was not significant at either of the tested CO_2 levels (Fig. 3). The effect of the increased

Fig. 1 Effect of different CO_2 and temperature range combinations (18 to 22, 22 to 26 and 26 to 30 °C) on the development of *Uromyces appendiculatus* on bean cv. Edna. Values with the same letter are not significantly different, according to Tukey's Test ($p < 0.05$). The mean of three experiments with a standard error of 2.2121
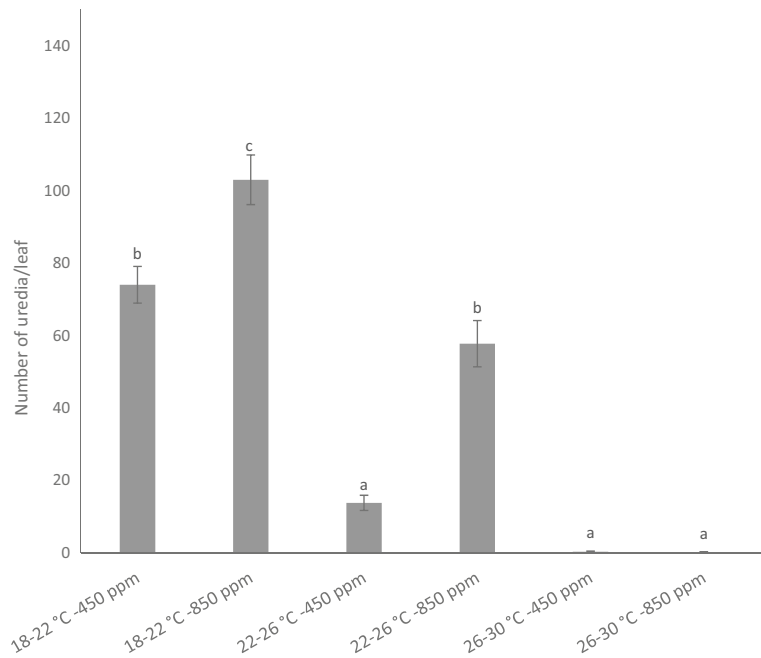
concentration of CO_2 on pelargonium rust severity at 14 to 18 and 18 to 22 °C was significant (Figs. 4 and 5).

Discussion

Research conducted over the past decades has shown how global climate change is/changes are associated with higher concentrations of atmospheric carbon dioxide, rises in temperature and extreme weather events. Considering the current rate of increase of CO_2 of 2 ppm per year, it is expected that CO_2 will surpass 500 ppm by the year 2067, but with the still steadily increasing CO_2 emission rates, it can almost be expected that CO_2 will surpass that value faster (Peters et al. 2012). For this reason, concentrations of CO_2 of between 500 and 1000 ppm should be considered as representative experimental values to study plant behavior, in the context of atmospheric CO_2 concentrations for the end of this century, as reported in others studies (von Tiedemann and Firsching 2000; Coakley et al. 1999; Gautam et al. 2013).

In the presence of the already increased temperature and CO_2 values in many geographic areas, and

Fig. 2 Effect of different CO_2 and temperature range combinations (14 to 18, 18 to 22 and 22 to 26 °C) on the development of *Uromyces appendiculatus* on bean cv. Edna. Values with the same letter are not significantly different, according to Tukey's Test ($p < 0.05$). The mean of three experiments with a standard error of 2.5351
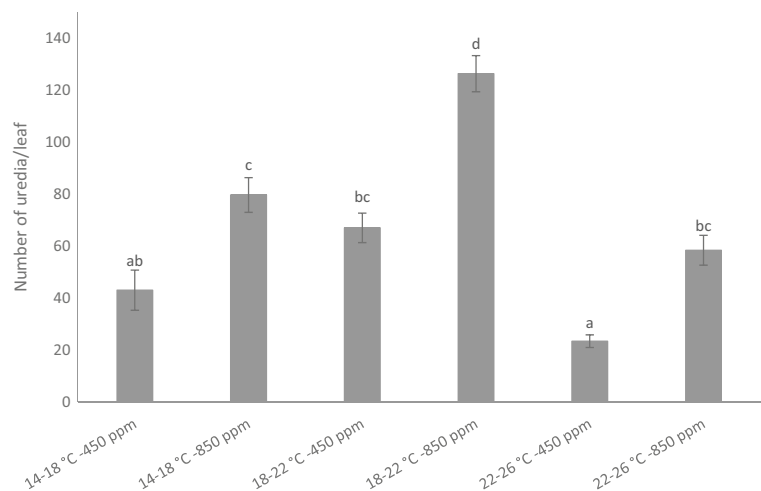
Fig. 3 Effect of different CO_2 (ppm) and temperature range combinations (18 to 22, 22 to 26 and 26 to 30 °C) on the development of *Puccinia pelargonii-zonalis* on pelargonium cv. Pac® Bergpalais Ⓟ. Values with the same letter are not significantly different, according to Tukey's Test ($p < 0.05$). The mean of three experiments with a standard error of 0.4935
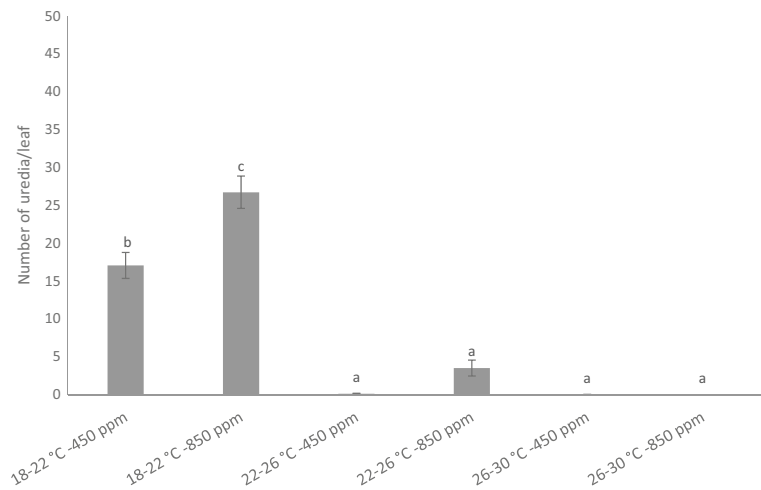
considering the prevision of future changes, researchers are more and more interested in evaluating how plants and their pathogens will react to further changes. Different approaches can be followed to provide knowledge on the possible effects of climatic changes and plant disease severity in several pathosystems. Phytotrons, which offer the possibility of working under controlled environmental conditions, provide a good opportunity to evaluate the behavior of different pathosystems under different temperature and CO_2 combinations.

The effects of elevated carbon dioxide concentrations on plant diseases can be negative or positive, and in the majority of cases (the) disease incidence is increased (Manning and von Tiedemann 1995; Franks et al. 2013).

Other trials carried out under phytotrons have shown that elevated CO_2 does not influence zucchini powdery

mildew (Pugliese et al. 2012b), but positively increases powdery mildew on grapevine (Pugliese et al. 2010), as well as basil black spots and downy mildew (Pugliese et al. 2012a: Gilardi et al. 2016), when correlated with high temperatures. Furthermore, soil-borne fungi, such as *Fusarium oxysporum* f. sp. *lactucae,* are also positively influenced by rising CO_2 and temperatures (Ferrocino et al. 2013).

Increased concentrations of atmospheric CO_2 and higher temperatures have been shown to negatively affect rusts on cereal crops (von Tiedemann and Firsching 2000). The positive effect of CO_2 and of rising temperature on the two rust pathogens has been evident in the present study (Fig. 5). The interaction between temperature and CO_2 was significant for both pathosystems ($p < 0.0001$). The effects of higher concentrations of atmospheric carbon dioxide and rises in

Fig. 4 Effect of different CO_2 and temperature range combinations (14 to 18, 18 to 22 and 22 to 26 °C) on the development of *Puccinia pelargonii-zonalis* on pelargonium cv. Pac® Bergpalais Ⓟ. Values with the same letter are not significantly different, according to Tukey's Test ($p < 0.05$). The mean of three experiments with a standard error of 0.5223
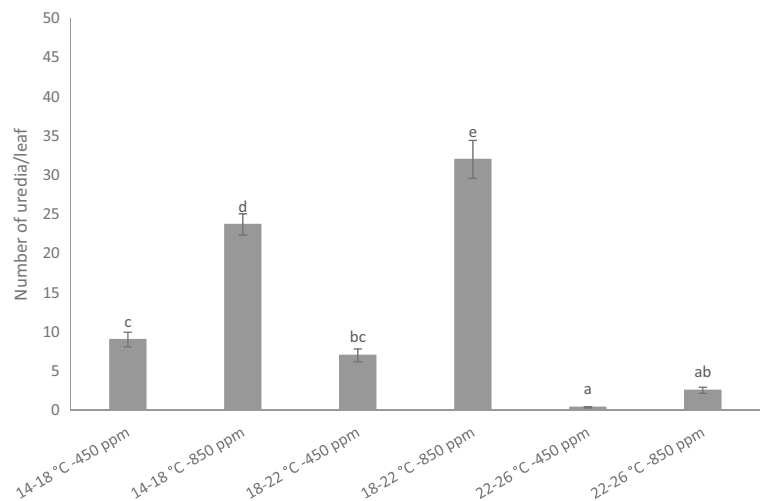
Fig. 5 Disease severity of
Uromyces appendiculatus on
bean cv. Edna and of *Puccinia
pelargonii-zonalis* on
pelargonium cv. Pac® Bergpalais
℗ under phytotrons at CO_2
ranging from 400 to 450 ppm and
800 to 850 ppm and temperature
ranging from 14 to 18, 18 to 22,
22 to 26 °C and 26 to 30
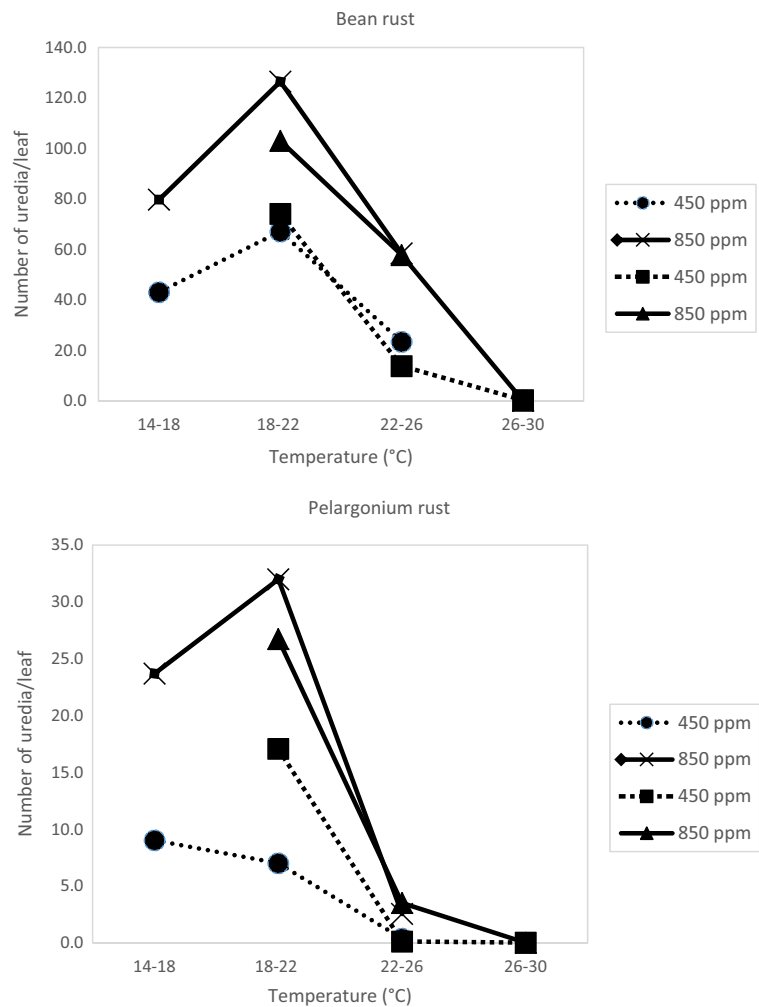
temperature on stomata density have not been evaluated in this study. However, the responses of different crop species to elevated concentrations of CO_2 may differ. Some species have shown a decrease in response to CO_2 enrichment, others an increase, and even others have shown no change in stomata density (Ferris and Taylor 1994; Hetherington and Woodward 2003; Franks et al. 2013). Moreover, there is evidence of variations in the degree of stoma recognition during the infection process within a crop species, and good efficiency was reported for bean rust (Wynn 1976). In the tests carried out under controlled conditions by Woodward and Kelly (1995), both *Phaseulos vulgaris* and *Pelargonium x hortorum* showed slight variations in stomatal density, which has been reported to vary from an initial number of 300 to a final value of 280 mm^{-2}, and from an initial number of 158 to a final value of 154 mm^{-2}, respectively.

Moreover, the efficiency of rust infection can also be affected by morphological features, such as the leaf surface properties and crop architecture. In the present study, *Uromyces appendiculatus* and *Puccinia pelargonii-zonalis* have been found to be positively affected by CO_2, in agreement with the results of other authors (Chakraborty et al. 2002), and this is probably linked to the increased production of plant biomass. Among the several types of vegetables, it has been shown that legume growth is improved under elevated CO_2 (Ainsworth and Long 2005).

The different temperature and CO_2 combinations, under the present experimental conditions, provided clear evidence of a possible increase in rust severity in the presence of higher CO_2 values, at the temperatures most favourable for rust development. The positive effect on rust severity was still observed at the highest

temperatures, when rust development was inhibited by these high temperatures. Unlike other rusts that are more resistant to higher temperatures, *U. appendiculatus* and *P. pelargonii-zonalis* will probably be affected negatively by global warming, and become more severe in cooler areas. Migration and adaptation to increasing temperatures are also possibilities for these species, as a consequence of climate changes (Chakraborty 2013).

The effect on rust severity of high CO_2 values could become more pronounced in the future, due to climate changes, and an increase in rust severity could be observed at temperature regimes less favourable to the pathogen.

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