

Effects of Elevated Carbon Dioxide Levels and Temperature on the Life History of the Madeira Mealybug (Hemiptera: Pseudococcidae)¹

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Abstract Atmospheric carbon dioxide concentrations and temperatures are increasing and, thus, the interactions between insect herbivores and their host plants in environments of elevated CO₂ concentration and temperature must be examined. We investigated the combined effects of elevated atmospheric CO₂ concentration (400 and 700 μmol mol⁻¹) and temperature (20, 25 and 30°C) on the development, survival and reproduction of two generations of the Madeira mealybug, *Phenacoccus madeirensis* Green, and the chemical composition of chrysanthemum, *Dendranthema x grandiflora* Kitam., leaves. The development of the mealybugs was temperature-driven and was not influenced by the CO₂ level or the number of generations. At higher temperatures, the duration to egg eclosion and the developmental time of adult females and males were significantly shortened. More eggs survived to adulthood at higher temperatures. Temperature had no influence on the egg eclosion percentage. The reproductive period of females was shortest at 30°C, while fecundity was highest at 20°C. There was a significantly higher proportion of females at the end of the experiment at lower than at higher temperatures. Elevated CO₂ level and temperature did not change the chemical composition (nitrogen and carbon concentrations, and carbon-nitrogen ratio) of the host plants. Relative water content of the leaf tissues was higher at 30°C than other temperature treatments. Our results show that the effects of temperature on the biology of the Madeira mealybug were stronger than that of the elevated CO₂ concentration.

Key Words Elevated carbon dioxide, mealybugs, rising temperature, plant-insect interactions

Continuous use of fossil fuels since the Industrial Revolution has increased the atmospheric carbon dioxide (CO₂) concentration from the pre-industrial level of 288 μmol mol⁻¹ to the current level of 365 μmol mol⁻¹ (Keeling and Whorf 1998). If the current trends of CO₂ input continue, the atmospheric CO₂ concentration in the next 50 yrs is projected at 700 to 1100 μmol mol⁻¹ (Houghton et al. 1996). Global warming resulting from the elevated level of CO₂ is expected to increase the temperature by 2 to 5°C (Houghton et al. 1996).

The performance of herbivores in an environment of elevated CO₂ generally depends on their modes of feeding (Bezemer and Jones 1998). Elevated atmospheric CO₂ concentration reduces performance of foliage feeders, leaf miners, and xylem

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feeders. The responses of whole-cell feeders (e.g., thrips and mites) and phloem feeders were inconsistent. Studies by Bezemer et al. (1999) and Hughes and Bazazz (2001) suggested that the responses of phloem feeders to elevated CO₂ levels vary with the species of insects and host plants, the biological levels (population or individual), and the experimental duration (single or multiple generations). Interactions between individuals and populations of insect and host plants in elevated CO₂ levels over multiple generations warrant further investigations.

Should the future temperature increase by 2 to 5°C as predicted (Houghton et al. 1996), studies investigating the effects of elevated CO₂ level alone would not provide an accurate prediction of the performance of insects. The combined effects of rising temperature and CO₂ level must be studied. However, all previous studies on the effects of elevated CO₂ on the biology of insects have ignored the temperature effects. Bezemer et al. (1998) conducted separate investigations into the effects of rising temperature and CO₂ levels on the biology of green peach aphids, *Myzus persicae* (Sulzer). No combined temperature and CO₂ treatment was included in Bezemer et al. (1998) studies.

Studies on insect herbivores in an environment of elevated CO₂ concentration also have implications in pest management. Plants grown at an elevated CO₂ level usually exhibit a "fertilization effect" (LaMarche et al. 1984). Yield and growth of the plants are enhanced due to the increased availability of carbon and the resulting stimulation of photosynthesis. These enhancing effects have been applied to commercial greenhouse ornamental production in the practice of CO₂ fertilization. CO₂ level is artificially increased to between 600 and 1200 µmol mol⁻¹ in some European and North American commercial greenhouses to stimulate the growth and yield of vegetable and ornamental crops (Wittwer 1986). The increases in atmospheric CO₂ level and temperature will affect crops, their pests, and the natural enemies (Stacey and Fellowes 2002). Mealybugs and other greenhouse pests commonly spread through greenhouses and nurseries by infested plant materials or cuttings. Because CO₂ fertilization is commonly used in propagation to stimulate growth of cuttings, the effects of such cultural practice on the population dynamics of insect herbivores in the greenhouses should be investigated to better predict pest status and to facilitate management decisions.

Most studies of the effects of elevated CO₂ level on phloem-feeders have been performed with aphids (Watt et al. 1995). The response of scale insects or mealybugs has never been studied. Here we report the investigation on the development, survival and reproduction of the Madeira mealybugs, *Phenacoccus madeirensis* Green, in an environment of elevated CO₂ concentration and temperature. To our knowledge, our study is the first attempt to study the combined effects of elevated CO₂ levels and temperatures on the biology of a mealybug species.

Materials and Methods

This study was conducted at the Georgia Envirotron, UGA/CAES/Griffin Campus, Griffin, GA. The Georgia Envirotron is a controlled environment research facility composed of multiple walk-in Conviron™ growth chambers (CG72, Controlled Environments Ltd., Winnipeg, Manitoba, Canada) with air temperature, relative humidity, photoperiod, and carbon dioxide concentration controls. In this study, the growth chambers were maintained at constant temperatures of either 20, 25 or 30°C, and CO₂ levels of either 400 or 700 µmol mol⁻¹. Each growth chamber was maintained at

a prescribed temperature and CO₂ level combination. A total of 6 chambers was used. The relative humidity and photoperiod in each chamber were maintained at around 68 to 75% and 16 h, respectively. The light intensity in the growth chamber was maintained consistently within the range of 630 to 650 micromoles/m²/s photosynthetic photon flux, which is similar to greenhouse conditions. CO₂ concentration in each growth chamber is controlled by the opening and closing of a solenoid valve that supplies CO₂ from a tank to the growth chamber through a manifold. The solenoid is operated by a microcontroller in each growth chamber equipped with a LI-800 infrared gas analyzer (IRGA) (LI-COR, Lincoln, NE).

Chrysanthemum (*Dendranthema x grandiflora* Kitam., cv. 'Pompona') cuttings were obtained from Yoder Brothers, Inc. (Barberton, OH). The cuttings were potted individually in 15-cm diam pots with Metro-Mix 300® potting medium (Scott-Sierra Horticultural Products Co., Marysville, OH). Approximately 10 g of Osmocote® 14-14-14 slow-release fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH) were applied to each pot after transplanting. The chrysanthemums were grown for 1 wk under greenhouse conditions before they were moved into the Envirotron. The chrysanthemums were allowed 2 wks to acclimate to the growing conditions in the Envirotron before mealybug infestation.

Six chrysanthemums were assigned to each growth chamber for the developmental biology study of the first generation of mealybug. One leaf from each chrysanthemum was chosen, marked with fluorescent fabric paint, and infested with an average of 28 eggs (ranged from 22 to 40). The eggs had been collected within 24 h from a colony of Madeira mealybugs maintained on coleus (*Coleus blumei* Benth., cv. 'Volcano'). The eggs were gently transferred onto the chosen leaves with a fine camel hair paintbrush. All the individuals on the same leaf constituted a cohort. Cohort development was observed daily. The duration to egg eclosion and to adult emergence and the survival rates of eggs and adults were recorded. The eclosion percentages of eggs were calculated as the number of eggs to successfully eclose divided by the total number of eggs used for initial infestation. Because the genders of the mealybugs could not be determined at the point of infestation, the survival rates to adulthood were calculated by dividing the total number of adults by the total number of eggs used for initial infestation.

Surviving adult females of the first generation were isolated individually in leaf cages and used in a fecundity study in their respective temperature and CO₂ combination treatment. The leaf cages were constructed from foam pads (25 x 25 mm) with a hole of 17 mm diam in the center. A screen with mesh size of 32 was glued over the hole of the foam pad. The leaf cages were clipped onto the leaf surfaces of the original host plants, with the adult females caged inside. After the females began ovipositing, the ovisacs were collected daily for egg counts. The duration of reproduction of a female was recorded as the period between the beginning and the end of oviposition.

Twelve plants were used in each growth chamber for the second mealybug generation. The plants were prepared in an identical procedure as those used in the first generation experiment. Each marked chrysanthemum leaf received an average of 20 eggs (range 16 to 24). The eggs were collected within 24 h from the first generation females used in the fecundity study. The developmental and reproductive biology of the cohorts in the second generation were studied as described in the preceding paragraphs. In the 20°C treatments at both CO₂ levels, the colonies were destroyed

during maintenance of the Envirotron. As a result, no data on the second generation of mealybugs at 20°C were collected.

Most studies on the effects of CO₂ levels on the biology of herbivores have suggested indirect enhancing effects of CO₂ levels on the nutritional status of the host plants (Bezemer and Jones 1998, Coviella and Trumble 1999). Thus, the nitrogen and carbon concentrations, and the relative water content of leaf tissues were analyzed at the end of the study. At the end of the experiment, the marked leaf and two other leaves of the approximately the same age and height on a plant were collected and sealed in a plastic bag. A method modified from van Iersel and Oosterhuis (1995) was used to determine the relative water content (RWC) of the leaves. The collected leaves were washed free of dirt and sooty mold and were then patted dry with tissue paper. Five leaf discs were excised from the interveinal tissue of each leaf using a cork borer (5 mm diam). The fresh mass (M_f) of the leaf discs was measured. The leaf discs were then floated in distilled water for 4 h to allow the leaf discs to absorb water. The leaf discs were dried with tissue paper at the end of the 4-h period, and the turgid mass (M_t) was measured. Dry mass (M_d) was determined after the leaf discs were oven-dried for 10 d. Relative water content was calculated from the equation

$$\text{RWC} = [(M_f - M_d) / (M_t - M_d)] \times 100\% \quad (1)$$

The leaf samples were then oven-dried and sent to Micro-Macro International Inc. (Athens, GA) for nitrogen and carbon concentration analyses. The reported measurements of the nitrogen content represented the total nitrogen content of the collected leaf tissues, i.e., reduced nitrogen and nitrate combined. The total nitrogen content of the dried leaf tissues was determined with a LECO CNS 2000 (LECO Corporation, St. Joseph, MI) (Mills and Jones 1996). Total phosphorous and potassium were determined by dry ashing and inductively coupled plasma spectrometry, using a Jarrell-Ash ICAP 9000 (Thermo Jarrell Ash Corporation, Franklin, MA) (Jones and Case 1990).

A regression analysis (PROC REG, SAS Institute 1985) was used to analyze the effects of temperature, CO₂ concentration and generation on the duration to egg eclosion and to adulthood, the eclosion percentage of eggs, the survival rates from egg to adulthood, the fecundity and the reproductive period of adult females, and the plant nutritional status (carbon and nitrogen concentrations, carbon/nitrogen ratio, and RWC). The eclosion percentage of eggs, survival rates to adulthood and RWC were arcsine-transformed to fit the data to a normal distribution before statistical analyses. All data collected for each biological parameter from each temperature × CO₂ combination were pooled and averaged before regression analysis. Initially, a quadratic regression model [Y = a + b(Temperature) + c(Temperature²) + d(CO₂) + e(Generation) + error] was used to describe the effects of temperature and CO₂ concentration on the biological parameters of the two generations of Madeira mealybugs and the host plants measured in this study. When the quadratic regression analysis showed no significant effect of the quadratic term (Temperature²), a linear model [Y = a + b(Temperature) + c(CO₂) + d(Generation) + error] was fitted to the same data.

Results and Discussion

The duration to egg eclosion and for nymphal developmental period of both male and female Madeira mealybugs were shorter at higher temperature, but were not

influenced by the CO₂ levels and generations (Fig. 1). Eggs eclosed in about 13 d at 20°C, which was about twice as long as the duration at 30°C (Fig. 1A). On average, an adult female emerged in about 20 d at 30°C, 28 d at 25°C, and 47 d at 20°C after infestation (Fig. 1B). Adult males emerged 1 to 9 d after female emergence (Fig. 1C). Males develop through 4 nymphal stadia before becoming adults, as compared to only 3 nymphal stadia in females (McKenzie 1967).

The treatments had no effect on the average eclosion percentage of the eggs, which ranged from 52 to 78% among the different temperatures, CO₂ levels and generations (Fig. 2A). The survival rate of the eggs to adulthood was best described by a linear regression to temperature, with no significant effects of CO₂ and generation treatments (Fig. 2B). The lowest average survival rate to adulthood (11%) was recorded at 20°C, and the highest average survival rate (49%) was observed at 30°C.

The highest proportion of female mealybugs (0.85) was recovered at 20°C, which was almost twice the proportion at 30°C (Fig. 3). These females at 20°C also demonstrated a longer reproductive period than the females at 30°C, while no significant effects of CO₂ and generations on the length of reproductive period were observed (Fig. 4A). Fecundity of female Madeira mealybugs was influenced by temperature and did not differ significantly among the CO₂ levels and generations (Fig. 4B). The fecundity was highest at 25°C with a female Madeira mealybug producing an average of 460 eggs in about 9 d, or on average 51 eggs per day.

Rising temperature and CO₂ levels generally did not have significant effects on the plant nutritional parameters measured in our study, except for the relative water content (Fig. 5). Nitrogen concentration in leaf tissues ranged from 8 to 31 mg g⁻¹ (on average 17 mg g⁻¹; Fig. 5A). The average leaf carbon concentration was 460 mg g⁻¹ (Fig. 5B), and the average C/N ratio was 28:1 (Fig. 5C). The relative water content of chrysanthemum leaves increased from about 90% at 20°C to 94% at 30°C (Fig. 5D).

The results of our study suggested that an increase in temperature has a greater impact on the development, survival, and reproduction of mealybug than elevated CO₂ level. In the future CO₂-rich environment and in greenhouses practicing CO₂ fertilization, the development of mealybugs is mostly temperature-driven in that elevated temperature increases Madeira mealybug abundance. This conclusion is similar to that reached by Bezemer et al. (1998) with aphids. The effects of elevated CO₂ level up to 700 μmol mol⁻¹ on the development of mealybugs are not significant in our study and was possibly masked by the stronger effect of higher temperature. However, it is possible that at a level most commonly used in CO₂ fertilization (1200 μmol mol⁻¹), the effects of elevated CO₂ level on insect development may be more prominent.

At higher CO₂ levels, the nitrogen content in plant tissues often decreases, and this nitrogen dilution effect was suggested to be responsible for the reduced performance of insect herbivores (Stiling et al. 1999, Joutei et al. 2000). Studies conducted on aphids (Awmack et al. 1996, 1997, Bezemer et al. 1998) have shown that the performance of this phloem feeder was enhanced when they fed on host plants grown in elevated atmospheric CO₂. On the other hand, other studies (Bezemer et al. 1999, Newman et al. 1999) reported no significant effects or reduction in population sizes of phloem feeders in an environment of elevated CO₂. No enhancement in the performance of the Madeira mealybugs, as measured by duration of development, survival and fecundity, was observed in our study. At the same time, we did not observe significant differences in the leaf tissue's chemical composition of plants grown at the two CO₂ levels. The elevated CO₂ concentrations in our study did not affect the

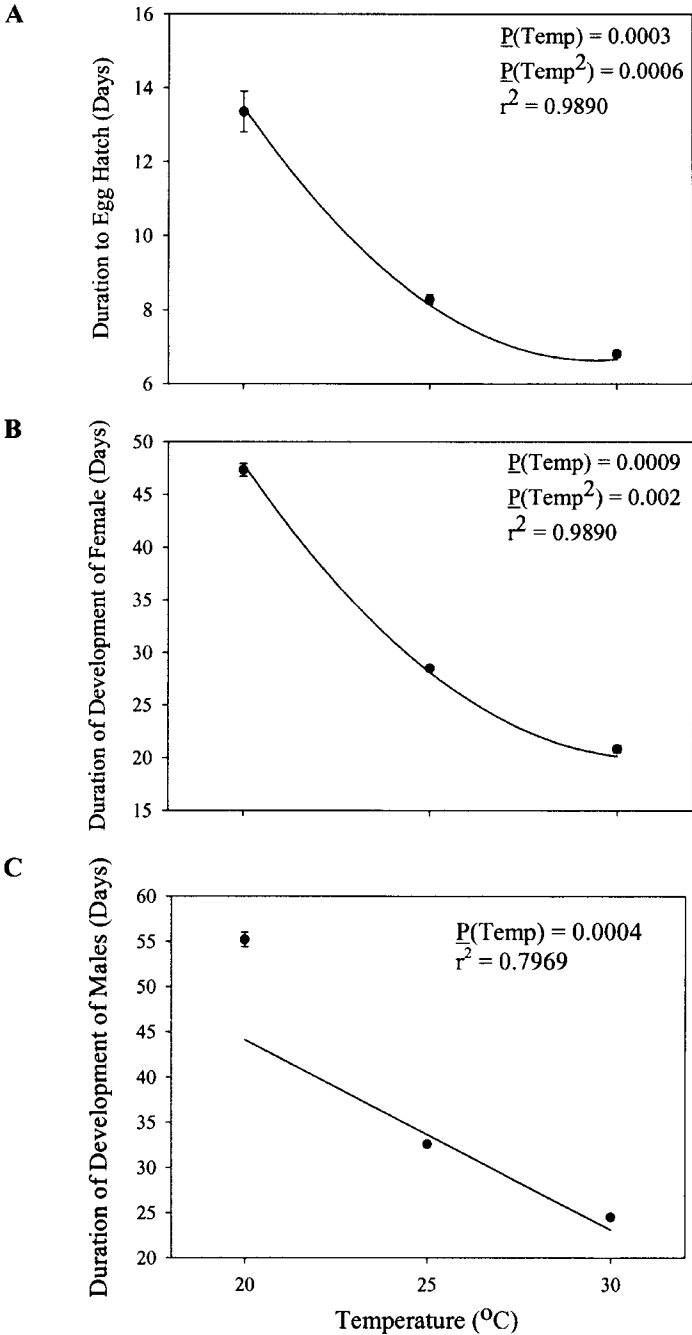


Fig. 1. Mean (\pm SE) duration to *P. madeirensis* egg eclosion (A) and total duration of development of females (B) and males (C) at the three temperatures. The main effects of CO₂ and generations were not significant. Lines represent the results of regression analyses. P-values presented are those of the temperature treatment.

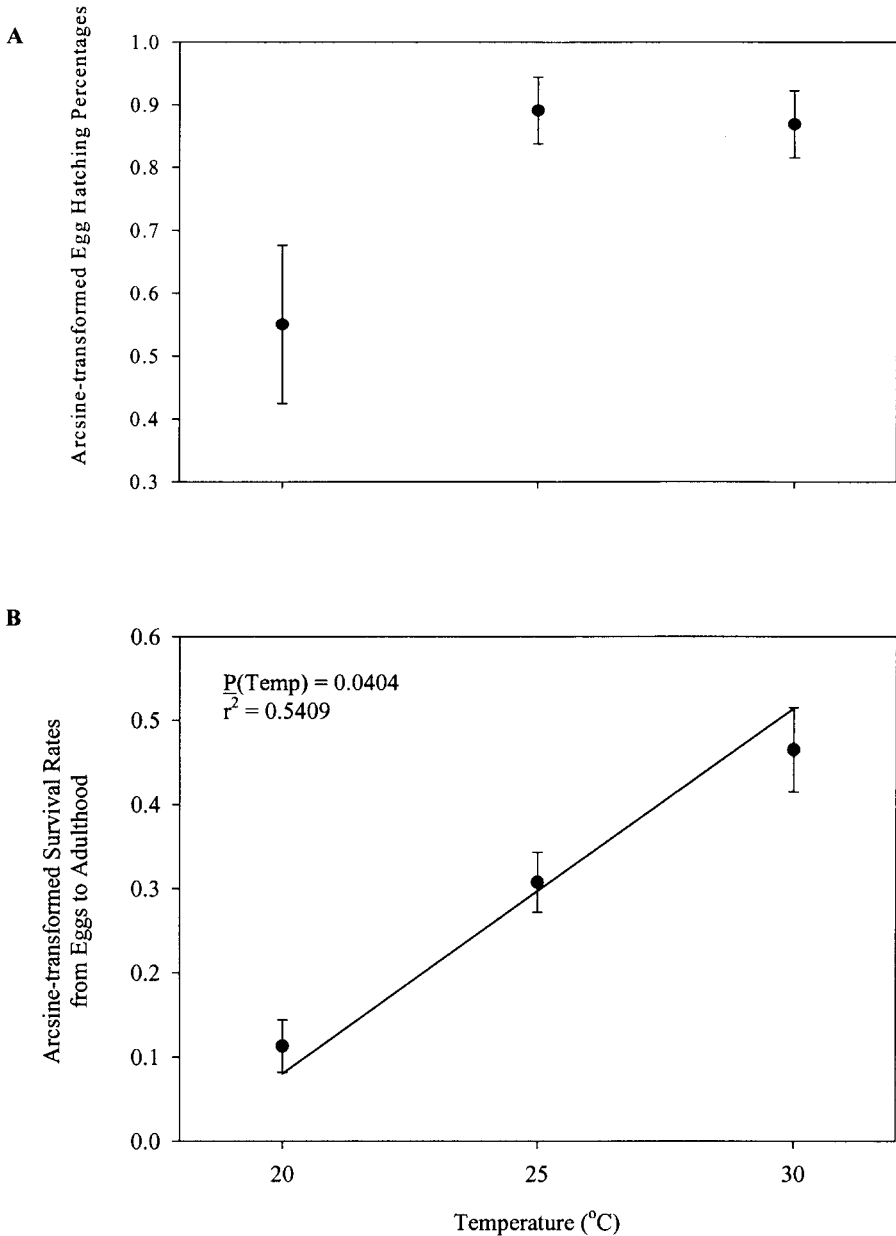


Fig. 2. Arcsine-transformed mean (\pm SE) egg eclosion percentage (A) and survival rate to adulthood (B) of *P. madeirensis* at the three temperatures. The main effects of CO₂ and generations were not significant. Line represents the result of linear regression analysis. P-value presented is that of the temperature treatment.

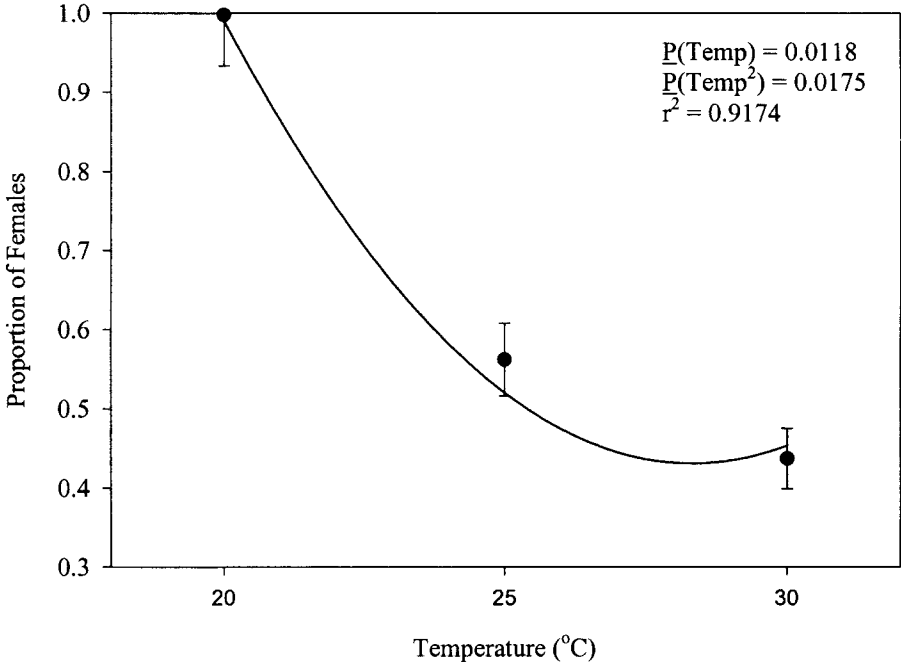


Fig. 3. Arcsine-transformed mean (\pm SE) proportion of *P. madeirensis* females at the three temperatures. The main effects of CO₂ and generations were not significant. Line represents the result of regression analysis. The p-value presented is that of the temperature treatment.

chemistry of the host plants, which may explain the lack of a CO₂ effect on the performance of the Madeira mealybugs.

Reduced performance of arthropods in elevated CO₂ was shown in other studies to be more severe in the later generations than the founding generations. The spittlebug *Neophilaenus lineatus* L. showed consistent reduction in survival and developmental rates over three generations (Brooks and Whittaker 1999). The establishment of the second generation of the two-spotted spider mite, *Tetranychus urticae* Koch, at elevated rather than at ambient CO₂ concentration was less successful (Joutei et al. 2000). They suggested that the reduced host plant nutritional value was the main cause for the reduced performance of the herbivores. No conclusion on responses emerges from the studies on multiple generations of phloem feeders. Multiple-generation (with experimental period >4 wks) studies on aphids have shown that in elevated CO₂ level, the populations of aphids either decreased (Newman et al. 1999), did not change significantly (Bezemer et al. 1999), or increased (Bezemer et al. 1998). The biology of the 2 generations of mealybugs in our study did not differ significantly.

When considering the effects of changing global climate on the biology of insect herbivores and its implications in pest management, the interactions between the ambient CO₂ level and temperature, and the feeding modes of the herbivores, should

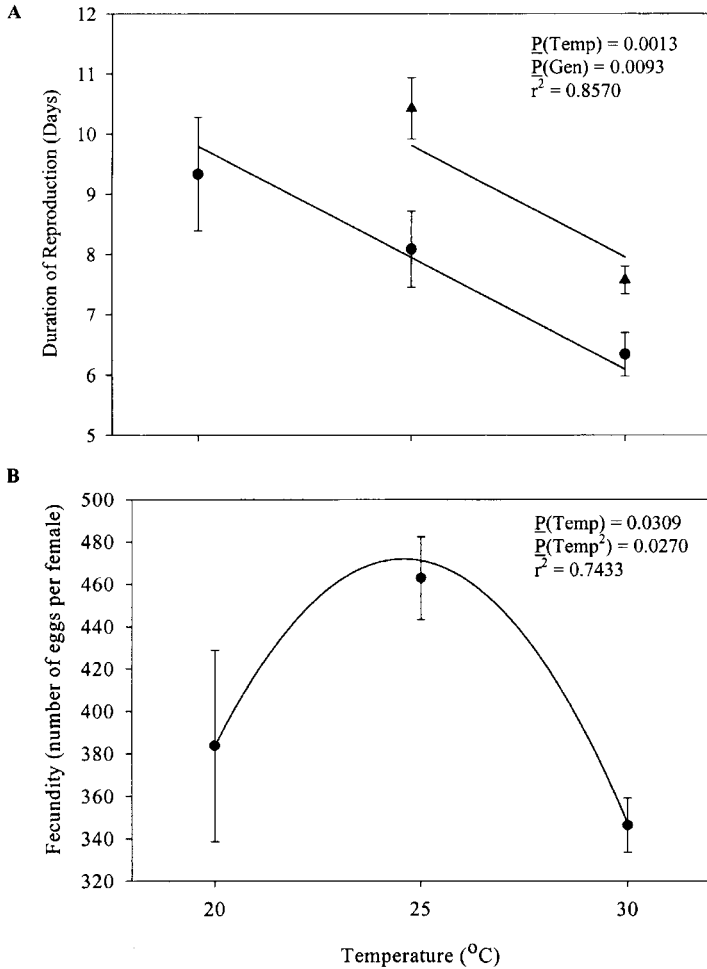


Fig. 4. Mean (\pm SE) duration of reproduction (A) and fecundity (B) of *P. madeirensis* females at the three temperatures. The main effect of CO₂ on the duration of reproduction was not significant, thus graph A is grouped by temperature and generations (● first generation; ▲ second generation). The main effects of CO₂ and generation on fecundity were not significant. Lines represent the results of regression analyses. The P-values presented are those of the temperature and generation treatments.

be the main concerns. Rising temperature may have a stronger effect in enhancing the performance of the mealybug, and possibly other phloem feeders, than the elevated CO₂ levels. However as evident from the varying conclusions of studies on the effects of CO₂ on arthropod herbivores of other feeding modes, arthropods may respond differently to the combination of elevated CO₂ levels and temperature. More

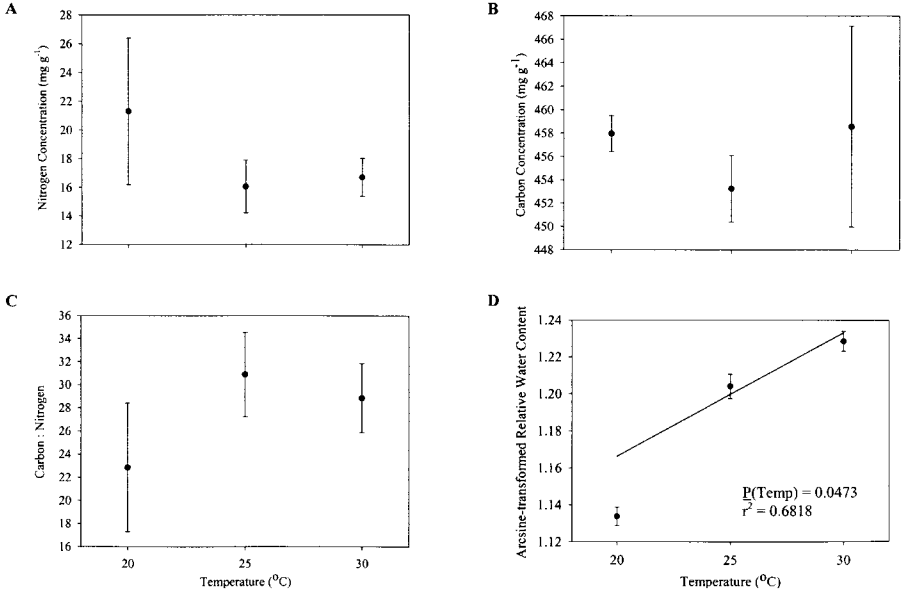


Fig. 5. Mean (\pm SE) nitrogen concentration (A), carbon concentration (B), carbon-nitrogen ratio (C), and arcsine-transformed relative water content (D) of chrysanthemum leaf tissues at the three temperatures. All treatments were not significant for all measured plant nutritional parameters, except for temperature on relative water content. Line represents the result of linear regression analysis. P-value presented is that of the temperature treatment.

studies on the interactions of elevated CO₂ level and temperature are needed in order to reach a generalization on the performance of arthropod herbivores.

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