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Tolerance of soil-dwelling Collembola to high carbon dioxide concentrations

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Carbon dioxide, tolerance, soil inhabiting Collembola

Abstract. The tolerance of soil inhabiting Collembola to high carbon dioxide concentrations has been assessed in two collembolan life-forms: the surface-dwelling species *Allacma fusca*, *Orchesella cincta* and *Tomocerus flavescens* as well as *Folsomia candida*, a species from deeper soil layers. Behavioural changes within the former group could be observed during short-term exposure (one hour) to 5 and 10% CO_2, respectively. In contrary, the limit of the tolerable carbon dioxide concentration of the latter species was only reached at 25% CO_2. Long-term tolerances expressed as survival times of the half number of specimens in 10% CO_2 ranged from a few hours (*A. fusca*, *T. flavescens*) to a few days (*O. cincta*) and > 6 weeks in the case of *F. candida*. These variations in tolerance may be a consequence of different environmental conditions arising from the risk of the species to experience hypercarbia. Although the ability of adult *F. candida* to survive hypercarbic periods is remarkable, chronic exposure to enriched CO_2 concentrations increased both the duration of egg development and the juvenile mortality rate.

INTRODUCTION

The gas composition of subterranean air is often higher in carbon dioxide than the normal atmosphere above ground. Occasionally, high metabolic activities of soil organisms accompanied by restricted diffusive exchange of the gases between the semi-secluded soil habitat and the open atmosphere may raise the carbon dioxide concentration to exceed 10% (Killham, 1994).

Many soil microarthropods, such as Collembola, live within the air-filled spaces between soil particles and have to cope with periods of air enriched by CO_2. Therefore, it can be expected that ecophysiological adaptations should exist to survive such extreme environmental conditions. Hitherto, the assessment of the tolerance of soil-dwelling animals to high carbon dioxide concentrations has attracted little attention. Pioneer work was done by Kupka & Schaerffenberg (1947), who reported on survival rates of large soil invertebrates in pure carbon dioxide. Ruppel (1953) first tested the short-term behaviour of two surface-dwelling and one edaphic collembolan species exposed to increasing carbon dioxide concentrations. Zinkler (1966) found in short-term experiments that the differences in CO_2 tolerance of 12 collembolan species from different habitats agreed in most cases with the known biology of the animals. The euedaphic *Onychiurus fimatus* does not lose its ability to maintain normal movements in air until 22% CO_2 is reached. Moursi (1962) recorded the lethal gas concentrations for 14 species of soil arthropods from forest soil, plant compost and cow dung after CO_2-exposure. In all cases high CO_2 tolerance relate well with species living in the cattle dung habitat. Recently, Holter (1994) investigated the tolerance of certain dung insects to increasing carbon dioxide concentrations. The animals moved normally in remarkably high concentrations up to 30 minutes.

Carbon dioxide concentrations in soil generally increase with depth. Collembola appear to be very suitable for comparative experiments of CO_2 tolerances. This group contains both surface-dwelling species and inhabitants of deeper soil layers. The aims of this study were to assess the short-term and long-term tolerances of four soil inhabiting collembolan species to high carbon dioxide concentrations and to examine the effects on their population dynamics during chronic exposure. Conclusions could be drawn concerning the different life history strategies which have evolved in these species.

MATERIAL AND METHODS

The Collembola used were three large surface-dwelling species *Allacma fusca* (L.), *Tomocerus flavescens* (Tullberg) and *Orchesella cincta* (L.) as well as the small edaphic species *Folsomia candida* (Willem). Adults of the first two species were collected from the upper litter layer of a deciduous forest (Kalwes) close to Ruhr-Universität Bochum, Germany. Specimens of *O. cincta* were derived from a culture maintained at the Vrije Universiteit of Amsterdam, The Netherlands. The animals were stored overnight in small plastic boxes with a layer of moistened plaster in the bases. As food, a suspension of *Pleurococcus* spp. (green algae) was offered. Adult individuals of *F. candida* were selected from parthenogenetic stock cultures which were reared in our laboratory for several years in plastic boxes on a plaster/charcoal (10 : 1) substrate. A standard mixture of powdered carrots, lucerne meal, Baker's yeast, agar and water was provided for food (Spahr, 1981).

All animals for study were stored at 18°C with food until used. Only individuals in good condition were exposed to various CO_2 concentrations. A schematic drawing of the experimental apparatus is shown in Fig. 1. Specially made glass tubes (approx. 120 ml) served as animal chambers. The bottom of the treatment chamber (Fig. 1, inset) was covered with a layer of moistened plaster/charcoal (10 : 1) substrate. Experimental animals were transferred to the test chamber and provided with food. The chamber was flushed with the CO_2-concentration to be tested at room temperature. The gaseous mixtures were delivered by a gas-mixing pump (Wösthoff 2M 301/a). After gassing (10 minutes, flow rate 210 ml/min) the treatment chamber was closed and made air-tight by straight-bore stopcocks and transferred to a climate room (18°C).

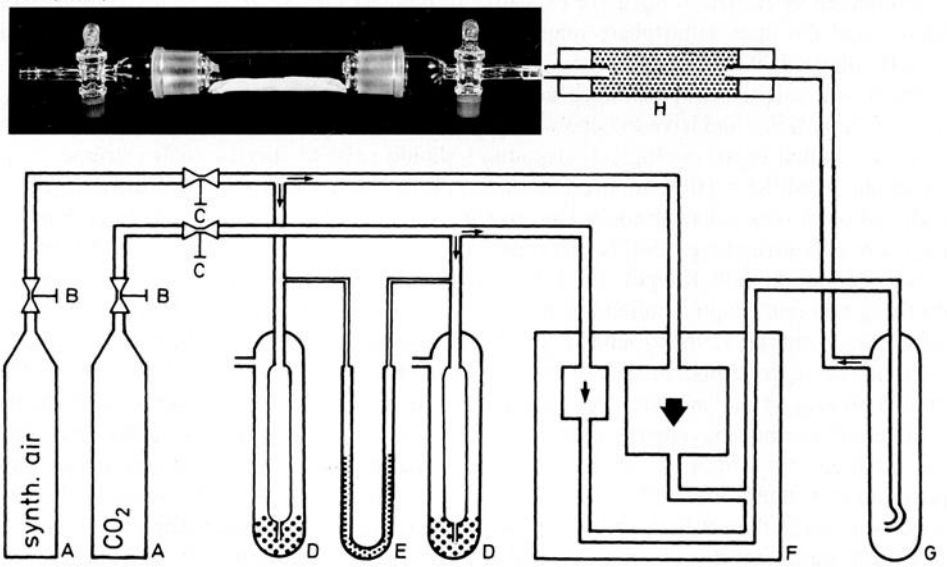

Fig. 1. Schematic diagram of the experimental apparatus to assess CO_2 tolerances of soil-dwelling Collembola. Gas cylinder and outlet valves (A, B, C), gas-mixing pump (D, E, F, G) and humidity regulator (H) connected to the animal treatment chamber (photographic inset).

Short-term tolerance was assessed for 20 specimens of each of the four species which were exposed individually to a range of gas concentrations (5, 10 and 25% CO_2). The CO_2 gas mixture under test was scored as tolerable for 1 h if all animals were able to maintain well-coordinated leg movements, quick reverse reactions and normal jumping behaviour. The behaviour of the animals was observed regularly every 15 minutes. Long-term tolerance of four collembolan species to increasing CO_2-concentrations (3, 5 and 10%) was assessed as the survival time of half the number of specimens under test. Only animals which behaved normally at the end of the experiment or recovered during 24 hours were considered as survivors. Effects of chronic exposure to 10% CO_2 on population dynamics of *F. candida* were studied for 40 days. The number of adults, eggs and juveniles was estimated every second or third day after CO_2 exposure. Juveniles were distinguished into three size class categories, namely < 1 mm, < 1.5 mm and >1.5 mm body length.

The CO_2-mixture within the animal chambers was renewed each week. The reliability of the carbon dioxide gas mixtures produced and the tightness of the test chambers during this period were confirmed by mass spectrometric control measurements (Varian M 3).

Oxygen consumption rates of pairs of Collembola were measured under normoxic conditions with a highly sensitive Warburg apparatus (Zinkler, 1966). Data are presented as mean ± SD. Data sets have been compared with a t-test at the 95% level of confidence to determine significant differences.

RESULTS

Short-term tolerance to increasing CO_2-concentrations

The limit of tolerable carbon dioxide concentration of the four collembolan species was 5% for *A. fusca* and *T. flavescens*, 10% for *O. cincta* and 25% in the case of the edaphic *F. candida*. The critical gas concentration was always indicated by behavioural changes. During the first few minutes of gas flushing the animals showed a number of escape reactions. 1–2 minutes later they became more or less immobile and rested in a typical bent body shape with inclined head and retracted legs. Exposure to higher concentrations caused virtual paralysis. Consequently, most of the animals lay on their sides.

Long-term tolerance to increasing CO_2-concentrations

Table 1 relates long-term tolerances of the four collembolan species to a range of carbon dioxide concentrations. Tolerance is expressed as the survival time of the half number of specimens under test. The three surface-dwelling species were strongly affected by both low and high CO_2 concentrations within a few days or < 24 hours, respectively. The relation between decreasing survival time and increasing carbon dioxide concentration was accompanied by a remarkable prolongation of the recovery period. In contrast, upper limits for carbon dioxide were comparatively high for *F. candida*. Survival times of animals exposed to 3, 5 or 10% CO_2 generally exceeded 6 weeks. Survival rates of about 50% occurred only during 3 day exposure to 25% CO_2. Stepwise acclimated animals to lower CO_2 concentrations seem to reveal an improved tolerance (data not shown).

TABLE 1. Tolerance of four collembolan species to various carbon dioxide concentrations expressed as survival time (days) of half the number of individuals (n = 8). All control animals exposed to normal air survive these conditions.

CO_2 concentration (%)	3	5	10
Allacma fusca	1.7	1.0	0.4
Tomocerus flavescens	14.0	2.7	0.8
Orchesella cincta	> 14.0	10.0	4.0
Folsomia candida	> 40.0	> 40.0	> 40.0

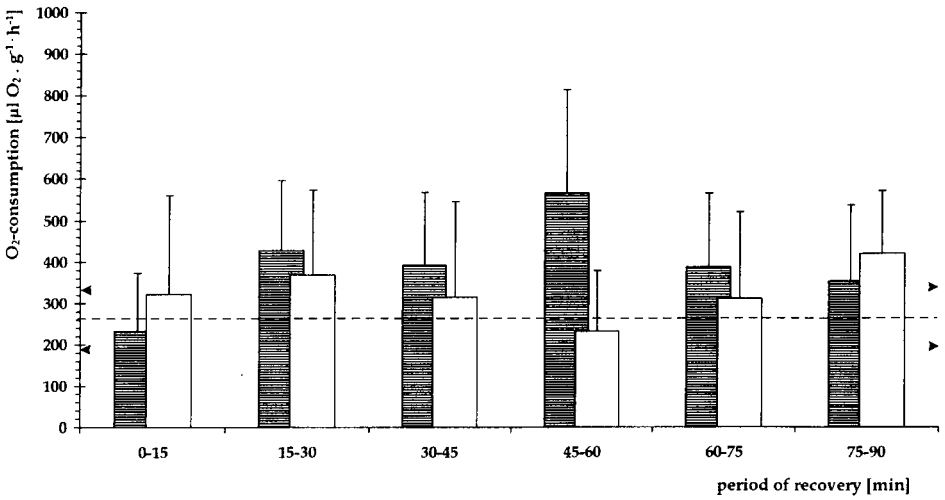

Fig. 2. Mean metabolic rates of *T. flavescens* at 18°C during 90 minutes of recovery from exposure to 10% CO_2 for 2–5 h (black columns, n = 17) and 24 h (white columns, n = 8), respectively. The broken line indicates the mean rate of oxygen consumption of untreated animals, and the black arrow heads show ± SD of this mean.

Metabolic effects of long-term CO_2-exposure

Oxygen consumption measurements were performed in order to characterize effects of long-lasting CO_2-exposure in terms of changes in metabolic activity. Since it was impossible to estimate the oxygen uptake of the three collembolan surface-dwellers in the presence of high CO_2 concentrations, their metabolic rates were measured during the first 90 minutes of recovery after exposure.

Unexpectedly, the oxygen consumption of *T. flavescens* did not differ significantly between control animals at rest and animals previously exposed to 10% CO_2 (Fig. 2). Furthermore, no significant differences were observed between short-term (2–5 h) and long-term (24 h) exposure nor in relation to the duration of recovery. Similar results were obtained after exposure to 3 and 5% CO_2 and in the case of *A. fusca* and *O. cincta* (data not shown). The results imply that energetic deficiencies can be excluded as a cause of the observed paralysis of the body appendages.

Effects of chronic CO_2-exposure on population dynamics

Since oxygen consumption measurements were unsuited for studying sub-lethal long-term effects of CO_2 on soil-inhabiting Collembola, latent effects on the population dynamics of chronic CO_2-exposure were elucidated. Treatment chambers (Fig. 1, inset), each containing 16 adults of *F. candida* and a separate portion of food, were flushed either with air enriched by CO_2 or with normal air (controls).

Figure 3 compiles the complex results of five single experiments concerning the population dynamics of 80 parthenogenetic adults after chronic exposure to 10% CO_2 over 40 days of observation. Eggs were laid by the animals during the first day. The fact that the glass tubes had to be held closed and air-tight during the whole incubation period made it

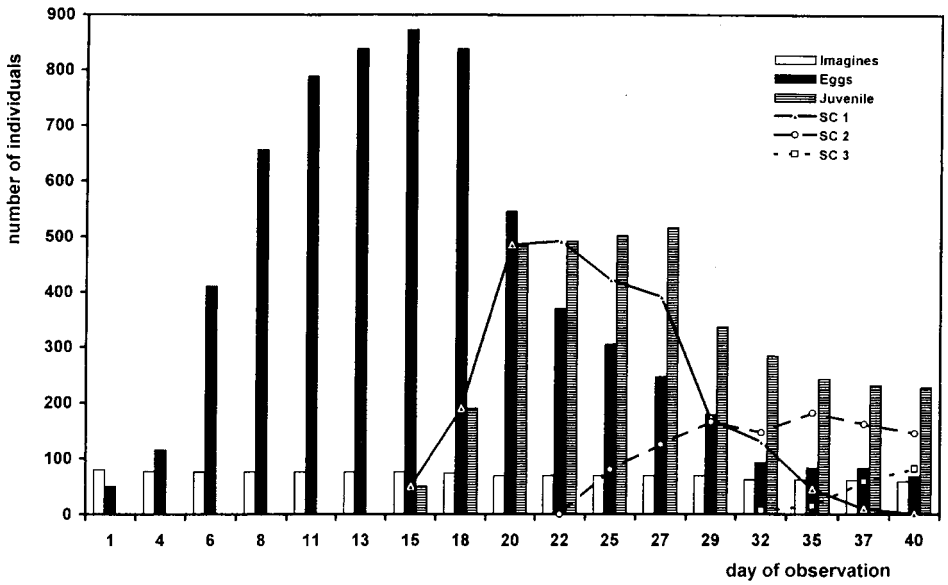

Fig. 3. Population dynamics of *F. candida* after chronic exposure to 10% CO_2 at 18°C expressed as the number of individuals with time. The compilation is of five single experiments, each with 16 adult individuals at the start. To discriminate juveniles of different age three arbitrary size classes (SC) were used.

impossible to separate the eggs produced. Therefore, only the total number of eggs present at the first and each subsequent day of observation were counted. The maximum number of eggs occurred at the end of the second week.

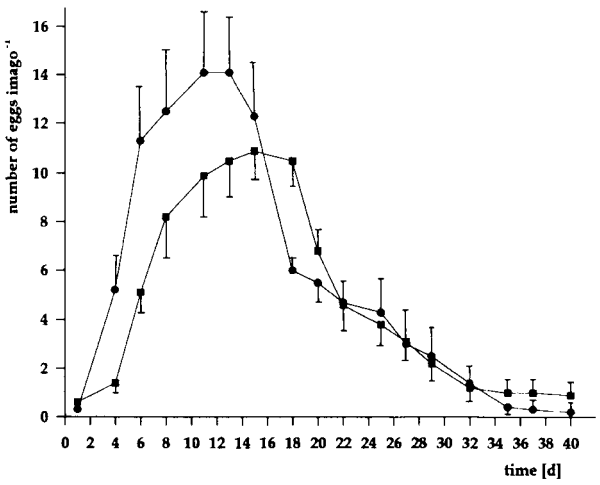

Fig. 4. Egg production of *F. candida* in relation to time. The animals were exposed to air enriched by 10% CO_2 (■) and normal air (●), respectively.

Fig. 4 shows a comparison between the egg production of *F. candida* maintained in normal air and under elevated CO_2 concentrations. The number of eggs per imago revealed no significant difference, but the egg laying process during the first 20 days seems to be decelerated during CO_2-exposure.

After chronic exposure to 10% CO_2 juveniles were observed for the first time at the end of the second week (Fig. 3). This implies that for the most part the number of days required for the embryonic development of *F. candida* eggs was 14 under these conditions. Soon

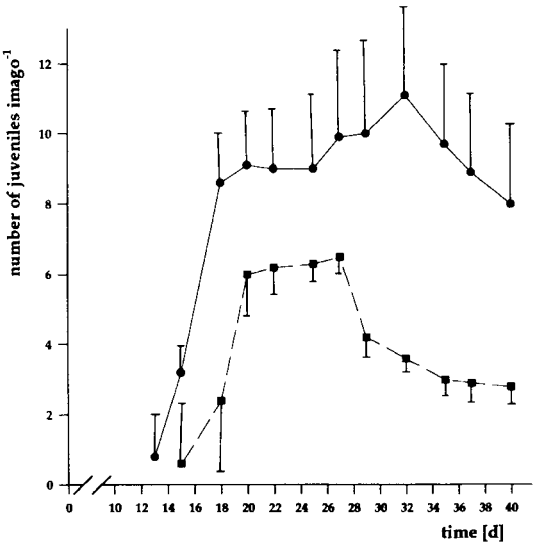

Fig. 5. Number of juveniles of *F. candida* in relation to time. The animals were exposed to air enriched by 10% CO_2 (■) and normal air (●), respectively.

after egg production and hatching begin to interact. The number of offspring reached a maximum at the 27th day. In order to discriminate the different ages of juveniles, three arbitrary size classes were introduced. As one would expect size class 1 was succeeded by size class 2 and the latter by size class 3.

Figure 5 presents the number of juveniles per imago in relation to time for those maintained in normal air and those under elevated CO_2 concentration, respectively. Clearly, the number of days required for the development of *F. candida* eggs from oviposition to eclosion increased in CO_2-enriched atmosphere. A delay of about 2 days was observed.

A second effect on population dynamics is revealed also by Figure 5. In normal air the number of juveniles per imago increased during the first 19 days, whereas in CO_2-enriched air this period is limited to 12 days. CO_2 seemed to exert deleterious effects on the viability of juveniles. The survival rate of juveniles during chronic CO_2-exposure was about 43%, whereas the survival rate of controls was significantly higher, namely 72%. Such an increased mortality rate was not observed in adult *F. candida* exposed to 10% CO_2.

CONCLUSIONS AND DISCUSSION

Carbon dioxide is classified as a nontoxic gas. Nevertheless, elevated CO_2 concentrations have long been known to cause deleterious effects in insects. High carbon dioxide concentrations induce behavioural changes in dung insects (Holter, 1994) or impair the egg-to-adult viability of certain *Drosophila* species (Bouletreau et al., 1984). High CO_2 concentrations immobilize insects. This popular anaesthetic in entomological research causes various delayed effects (Goto, 1971; Fuzeau-Braesch & Nicolas, 1981). High CO_2 levels are also used to kill and control insect infestations in stored food (Bailey & Banks, 1980).

Compared to the amount of work describing the effects of non-physiologically high CO_2 concentrations, little effort has been made to study environmental CO_2 variations. Many soil insects have to cope with periods of air enriched by CO_2. The respiratory gas concentrations of CO_2 in the microhabitats of some burrowing insects and within decaying stumps inhabited by termites rise as high as 6–8% (Anderson & Ultsch, 1987). Occasionally, the carbon dioxide concentration in particular soil microhabitats may exceed 10% (Killham, 1994). Dung pat concentrations of CO_2 are often above 20% (Holter, 1991).

Surface-inhabiting Collembola exhibit a remarkably lower limit of tolerable carbon dioxide concentration than species from deeper soil layers. This variation in tolerance may be a consequence arising from the risk to experience hypercarbia. Similar differences in CO_2 tolerance which agree well with the known biology were reported also for four dung insect species (Holter, 1994) and for 14 species of soil arthropods from forest soil, compost and cattle dung (Moursi, 1962).

An increase in CO_2, as high as 5%, causes behavioural changes in surface-dwelling Collembola. Escape reactions, non-coordinated leg movements and deferred reverse reactions were observed in the present study. Higher concentrations virtually paralyzed all the species examined. The animals resembled exhausted individuals after considerable jumping activity (Ruhfus & Zinkler, 1995) or during severe environmental oxygen deficiency (Zinkler & Rüssbeck, 1986). During the present experiments the springtails were exposed to comparatively high CO_2 concentrations accompanied by only minor decrements of O_2. The oxygen partial pressure of normal air and synthetic air enriched by 10% CO_2 is about 158 and 143 torr, respectively. *T. flavescens* became immobile only below 20 torr (Zinkler & Rüssbeck, 1986). Therefore, oxygen deficiency is not the cause of the observed paralysis of collembolan body appendages after exposure to high CO_2 concentrations.

We suggest that acid-base disturbances may be the cause for their collapse under excessive CO_2. Although body fluids are more or less buffered, the cells are rendered acidic due to the interconversion of CO_2 and bicarbonate. This acidification may be enhanced by the fact that Collembola rely on cuticular respiration.

The different tolerances to CO_2 of surface-dwellers and inhabitants of deeper soil layers relate well with the result that only *F. candida* is able to accumulate large amounts of lactic acid. A significant amount of 14.5 ± 1.7 µmoles lactate . g^{-1} dry weight (n = 3) was found after an anoxic period of 6 h (Zinkler & Rüssbeck, 1986). During recovery all the lactate is respired without any other effects. Obviously, the acid-base-balance of *F. candida* seems to be well adapted to sustain even a pronounced acidification.

The ability of adult *F. candida* to survive hypercarbic periods is remarkable. In contrast, chronic exposure to enriched CO_2 concentrations increased both the duration of egg development and the juvenile mortality rate. A comparison of these population dynamics effects with other findings is hampered by the fact that all studies on *F. candida* population growth reported in the literature were done in normal air. Therefore, only the population data concerning our controls can be directly compared.

Population dynamics effects found in the present study were observed at 18°C. This temperature is within the range of maximum fecundity of *F. candida* (Snider, 1973). Variation in fecundity of *F. candida* in the laboratory may be enormous. On average, a female lays a total of 167 eggs (Green, 1964) or 1011 eggs (Snider, 1973) during its lifetime. The present paper compared population development over a much shorter time period. During the 40 days of observation the largest number of eggs per imago was estimated to be 14.1 ± 2.5 (n = 5). These data suggest a similar rate of egg production as reported by Green (1964). The weekly egg production in the present study was only a quarter of that compared to the weekly oviposition rate of single kept animals over twelve weeks (Booth, 1983). Snider (1973) suggested that rearing techniques and differences in genetic strains may strongly influence the fecundity of *F. candida* in culture.

The number of days required by our experimental control eggs to complete embryonic development is in accord with the data given by Snider (1973). Chronic exposure to enriched CO_2 concentrations retarded the egg development of *F. candida*. Further studies of the population dynamics effects of combined high carbon dioxide and low oxygen concentrations, an environmental condition common in soils, would be helpful in the future.

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