Stressing food plants by altering water availability affects grasshopper performance

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Abstract.   Extreme weather events like drought and heavy rain are likely to increase with climate change in Central Europe and may affect nutrient content in plants. They may therefore influence the performance (growth rate, developmental time, mortality, body size/mass, fecundity) and population dynamics of herbivorous insects. We conducted a common-garden experiment on food plants to investigate effects of severe drought and moisture events on reproduction and fitness components in the insect herbivore *Chorthippus biguttulus* (Orthoptera, Acrididae). Periodic irrigations of food plants were used to simulate a 60% decrease in average summer precipitation (drought treatment), a 40% increase in average summer precipitation (moisture treatment) and a normal summer precipitation (control treatment). Individuals of *C. biguttulus* that fed on drought-stressed plants showed beneficial effects on life-history traits including an increased reproductive success than grasshoppers that fed on control plants. The opposite was true for individuals feeding on plants grown under severe moisture conditions. We propose that herbivore performance is influenced by increased concentrations of soluble proteins and amino acids in plants under drought stress conditions. Our results suggest that drought events may increase population performance and consequently population density in the grasshopper species *C. biguttulus*, while extreme moisture events may cause negative population trends.

Key words: amino acids; *Chorthippus biguttulus*; grasshoppers; performance; plant stress; soluble protein.

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INTRODUCTION

Predictions of future climate change in Central Europe assume that—besides increases in temperature and CO_2 concentration—the frequency and intensity of extreme weather events will increase, too (Easterling et al. 2000, Meehl et al. 2000, Salinger 2005, IPCC 2007). Changes of precipitation regimes have been observed in the past years in climate studies (Beck et al. 2001, Schonwiese et al. 2003, Schmidli and Frei 2005) and have been predicted for the future by various models (Raisanen and Joelsson 2001, Christensen and Christensen 2003, Sanchez et al. 2004,

Semmler and Jacob 2004). In addition to direct effects on animal populations these environmental influences could as well affect the interaction of plants and their herbivores (Mattson and Haack 1987, Coviella and Trumble 1999, Hunter 2001, Bale et al. 2002, Hale et al. 2003, Villalpando et al. 2009).

Important environmental influences on plants are severe drought and moisture events which can have strong effects on various plant compounds like the plants' nutrient content (e.g., protein content, amino acids) and secondary plant defenses (Hsiao 1973, Gershenzon 1984, Bannister 1986, Hoffmann and Parsons 1997).

Proteins and amino acids, especially proline, are expected to increase with drought stress in plants (Hsiao 1973). Secondary plant compounds should increase in plants under drought conditions (Gershenzon 1984). There is evidence that drought stress in plants increases insect survival, growth and reproduction through elevated plant nutrient levels, especially nitrogen and amino acids and lowered plant defenses (Rhoades 1983, White 1984, Mattson and Haack 1987, Hale et al. 2003).

Generally it is known that changes in plant compounds have large effects on the performance of herbivores (Wagner and Reiser 2000, de Bruyn et al. 2002, Holzer et al. 2003, Scheirs et al. 2003). Insect responses to changes in food plant quality include compensatory feeding (Fajer 1989, Docherty et al. 1996), decreased growth rates (Fajer 1989, Traw et al. 1996, Hättenschwiler and Schafellner 1999), reduced final body mass, prolonged developmental time (Goverde and Erhardt 2003), increased mortality (Fajer 1989), and reduced reproduction (Brooks and Whittaker 1998, Buse et al. 1998). At the level of the individual, these responses may negatively influence reproductive output and, as a consequence, population dynamics of insect herbivores. Observed changes in food plants which can affect herbivorous insects are often related to phenol content (plant defense) that reduces feeding and oviposition (Bernays and Chapman 2000, Dettner and Peters 2003). There is also much evidence that insect growth and reproductive success is often determined by a relative shortage of resources, especially of nitrogen (White 1993). Available nitrogen in terms of proteins is the basic material for soft tissues and the integument of insects. As nitrogen increases in food plants, insects convert more plant material into body tissue (McNeil and Southwood 1978). Beside nitrogen, water is a primary determinant of nutritional quality in insect life (Scriber 1984, Showler 2002). In the grasshopper species *Melanoplus bivittatus* (Orthoptera, Acrididae) a strong relationship between diet selection and grass quality was shown (Jonas and Joern 2008), indicating that plant defenses are not necessarily more important in food selection than nutrient composition. Furthermore, protein and amino acid content in food plants can affect different behavioral and phys-

ical characteristics of phytophagous insects (Behmer and Joern 1994, Scheirs et al. 2004, Zavala et al. 2004a, 2004b). Many studies have examined responses of insect herbivores to drought-stressed food plants (Mattson and Haack 1987, English-Loeb et al. 1997, Schowalter et al. 1999, Huberty and Denno 2004). However, in most of these studies, little attention was paid to effects on reproduction. Likewise little is known about moisture stress of plants and its influences on herbivorous insects.

Grasshoppers as representatives of herbivorous insects belong to the most important primary consumers in grassland ecosystems and serve as prey for higher trophic levels. It has been demonstrated experimentally that grasshoppers can affect plant populations and community dynamics by e.g., suppressing abundant, highly competitive grass species and thus facilitating the biodiversity in grasslands (Schmitz 2003). Therefore grasshoppers are suitable model organisms for studies concerning climate change and its possible effects on grassland communities.

In order to evaluate the importance of effects of climate change, i.e., drought and moisture events, we tested the influence of three different water treatments on food plant quality, on life-history traits including reproductive success of *C. biguttulus* grasshoppers. Because we do not know much about the effects of moisture stress in plants on the performance of herbivores but something more about their performance when feeding on drought-stressed plants, we investigated the differences in grasshopper performance between these two stress levels of food plants. Based on the fact that amino acids and soluble protein content increase in plants under drought stress, we hypothesize that grasshoppers feeding on drought-stressed plants show increased life-history traits compared to grasshoppers feeding on plants under severe moisture conditions and also under controlled conditions of plants.

Materials and Methods

Study organism

The experiment was conducted with two populations of the species *Chorthippus biguttulus* (Acrididae, Gomphocerinae; Linnaeus 1758), one

of the most abundant grasshopper species in Central Europe (Ingrisch and Köhler 1998, Maas et al. 2002). In August 2007, we caught 40 adult females from each of two different locations in northwestern Germany, near the city of Bielefeld. Individual females of *C. biguttulus* grasshoppers were kept separately in plastic terraria ($18 \times 11 \times 13$ cm) in the lab for three weeks. They were fed on a field-cut grass mixture. Each terrarium contained small plastic cups filled with a mixture of moist sand and soil (1:1) for oviposition. Four weeks after the last oviposition the plastic cups that contained the egg cases were transferred to a climatic chamber where they were stored at 4°C until the start of the experiment in May 2008.

Dietary mixing has repeatedly proved to increase fitness and decrease mortality in grasshoppers (Bernays et al. 1994, Unsicker et al. 2008). Therefore we used in all three treatment groups a combination of five food plant species—four species of the family Poaceae (*Agrostis capillaris*, *Dactylis glomerata*, *Festuca rubra*, *Poa pratensis*) and one species of the family Fabaceae (*Trifolium repens*).

Experimental design

A feeding experiment with three plant treatment groups was conducted in a greenhouse. The plant treatments were watered differentially. The Helmholtz Association of German Research Centres predicts a precipitation increased by 40% and a precipitation decreased to about 60% from spring to autumn in Germany in the years 2071–2100 as related to the period of 1961–1990. We simulated extreme events for the water availability during the summer. The control treatment represented an average German summer precipitation of 239 l/m^2 according to the German Meteorological Service. The drought stress treatment represented about 60% less precipitation then the control treatment whereas in the moisture stress treatment the soil was saturated with water once every day (about 40% more precipitation then the control). For all plant treatments water was applied daily at ground level. Hence plants of the drought stress treatment were watered daily—as well as the control and the moisture treatment—however with different water quantities.

Food plants were sown in mid of February 2008 in plastic containers ($60 \times 40 \times 15$ cm)

containing a soil mixture of vermiculite and 'Wesersand' (1:1). Half a gram of seeds were sown of each plant species on the same place in each container in order to avoid the plant species mixing. Each treatment group consisted of 15 plant containers. For three months plant containers were watered as required and once a week fertilized with 1 liter of a 50% solution of a modified Hoagland solution (KNO_3 0.006 mol, $Ca(NO_3)_2 \times 4H_2O$ 0.004 mol, $(NH_4)_2SO_4$ 0.001 mol, KH_2PO_4 0.001 mol, $MgSO_4 \times 7H2O$ 0.002 mol, KCL 0.001 mol, Fe-citrat $FeC_6H_5O_7$ and some trace elements: H_3BO_3, $MnSO_4$, $ZnSO_4$, $CuSO_4$, MoO_3) (Hoagland and Amon 1950). One week before starting the experiment plant containers were randomly allocated to the three treatment groups.

At the beginning of May 2008, egg cases were moved into a climatic chamber with a temperature of 26°C to allow hatching of the nymphs. All grasshopper nymphs hatched after 14 or 15 days and then were directly placed in wooden cages ($40 \times 27 \times 32$ cm) which were positioned above the plant containers of the experiment. On May 18 2008 after all grasshoppers had hatched, about 500 nymphs of each population were randomly assigned to the three treatment groups, to allocate each population of about 100 insects per treatment (20 grasshopper nymphs per cage, meaning 5 cages per treatment and population and each treatment combination five times replicated). In order to account for position effects and microclimatic variation, containers, cages and grasshoppers were rotated once a week, to make sure that every week each cage was placed in a different plant container (also at reserve containers) and location in the greenhouse. Each grasshopper group was transferred into another cage, and the plant containers were moved to another place, to guarantee that containers, grasshoppers and grasshopper groups were never at the same place. Grasshoppers could feed on the same type of plant treatment from hatching until three weeks after the final molt. No additional water was provided to grasshoppers, so that they received water only from food plants.

Temperature and humidity in the cages were measured and recorded over the complete time of the experiment. Both parameters differed between treatments only at night ground level

but at night grasshoppers usually were on the ceiling of the cages. We therefore assume that direct effects of temperature and humidity had negligible effects on grasshoppers and were under controlled conditions. A statement about the consumption of food plants of grasshoppers cannot really be given because this was difficult to observe in this experimental set-up. Plants were mostly wilting under moisture conditions and were discolored under drought stress. Plants reared under control were growing like under natural conditions.

Grasshopper fitness components and reproductive success

For each grasshopper we recorded the developmental time from hatching (the start of the experiment, meaning average hatching day of grasshopper nymphs) until the final molt. Grasshoppers were collected in the evening of the day they had completed their terminal molt and were individually marked by points of paint on the pronotum. On the following day, the adult grasshoppers were weighed and separated by sex but transferred again to the experimental cages in the greenhouse so that they could feed on the same food plant treatment they did before. Three weeks after the first sexually mature male grasshoppers appeared, they were placed with a randomly chosen single female of the same treatment in a small plastic terrarium (18 × 11 × 13 cm) in a climate chamber. The temperature in the climate chamber was 26°C for 12 hours at daytime and 18°C for 12 hours at night. The relative humidity in the climate chamber was kept at 40%. Throughout, grasshoppers were fed the same type of food plants as before. The food plants were placed in small plant pots with the same soil mixture as used for the plant containers and watered as in the experiment before. Males were left with females for 37 days. For oviposition, each female grasshopper was provided a small plastic tub filled with moist sand and soil (1:1). Then the experiment was terminated and grasshoppers were stored in pure ethanol. As an estimation of body size we measured the left femur length of adult grasshoppers after the adult molt with an ocular micrometer under a stereomicroscope. After the termination of the experiment the egg cases laid by each female grasshopper were counted, weight (scale: Kern

770) and buried in the soil again. Four weeks after the experiment was terminated, egg cases were moved into a climate chamber and kept at 4°C over the winter. To determine the hatching rate of offspring, egg cases were incubated at 26°C in a climate chamber starting 06 May 2009. Number of hatched offspring and number of eggs in the cases laid per female were counted.

Plant compound analyses

The five plant species were harvested once a week in each of the three treatment groups over a time period of five weeks to allow biochemical analyses of relevant plant nutrition compounds. Plants were non-flowering throughout the experiment. From all plant species and all plant containers a sample of leaves which were not damaged by grasshoppers was cut once a week and mixed for measuring the soluble protein content, amino acid concentration and secondary plant compounds. For determination of soluble protein content plants were frozen at −20°C in a freezer and after analysis we converted the values in dry mass. For determination of total amino acid concentrations cut plants were frozen in liquid N, then at −20°C in a freezer and for analysis lyophilized. To analyze the soluble protein content we followed the method of Bradford (1976). The extraction was done by a myrosinase buffer. Total amino acids and secondary plant compounds were analyzed using an Agilent 1200 HPLC system for chromatography (Agilent Technologies, Boeblingen, Germany) coupled to an API 3200 tandem mass spectrometer (Applied Biosystems, Darmstadt, Germany). Freeze-dried samples for amino acid analysis were extracted by methanol and 1,3-Dimethoxybenzene as internal standard. All measurements of plant compounds were based on dry masses of plants.

Statistical analysis

To analyze effects on grasshopper performance, Linear Mixed Effect Models (LME) and Generalized Linear Mixed Models (GLMM) were fitted to the data using R version 2.10.1. 'Treatment' (a factor with the three levels drought, control and moisture) and sex were used as fixed effects and 'group of grasshoppers' as a random effect to account for the non-independence of individuals living together in a

group. Body condition was modeled as body mass controlling for the effect of femur length (as a measure of structural size). Populations were pooled, except for developmental time, because no differences between populations were detected. GLMMs with Poisson error structure and log-link function were fitted to model the number of egg cases produced by individual females. GLMMs with binomial error structure and logit-link function were fitted to model the proportion of hatched offspring per female using the number of eggs per female as the binomial denominator. Non-significant terms were removed from the model. For plant analysis a two-way ANOVA without replication was calculated with 'treatment' and 'week' as fixed effects. Plant species were pooled because all species showed the same tendency in each of the treatments and responded in similar ways on water availabilities. All dependent data were tested for normality with the Lilliefors test and log or square root transformed if necessary. Tukey HSD-Tests were conducted for pairwise comparisons. We corrected for multiple testing by Bonferroni. As a correction for multiple testing did not change significance for most of the parameters, we do not give Bonferroni corrected p-values but note if there are no significance of p-values after correction. Means are always displayed with standard errors (SE).

RESULTS

Developmental time

Developmental time until final molt differed significantly between insects kept on plants that differed in treatment (LME: $F_{2,26} = 66.40$, $p < 0.001$, Fig. 1a), sexes (LME: $F_{1,365} = 24.90$, $p < 0.001$, Fig. 1a) as well as between populations (LME: $F_{1,26} = 4.80$, $p = 0.038$, Appendix: Table A1). We found significant differences between all three water treatments (Tukey HSD-Test: $p < 0.001$, except control vs. moisture $p = 0.001$ Fig. 1a). Individuals of the control treatment developed about 8% slower than individuals of the drought treatment. Individuals of the moisture treatment developed about 11% slower than individuals of the drought treatment and 4% slower than individuals of the control treatment (Appendix: Table A1).
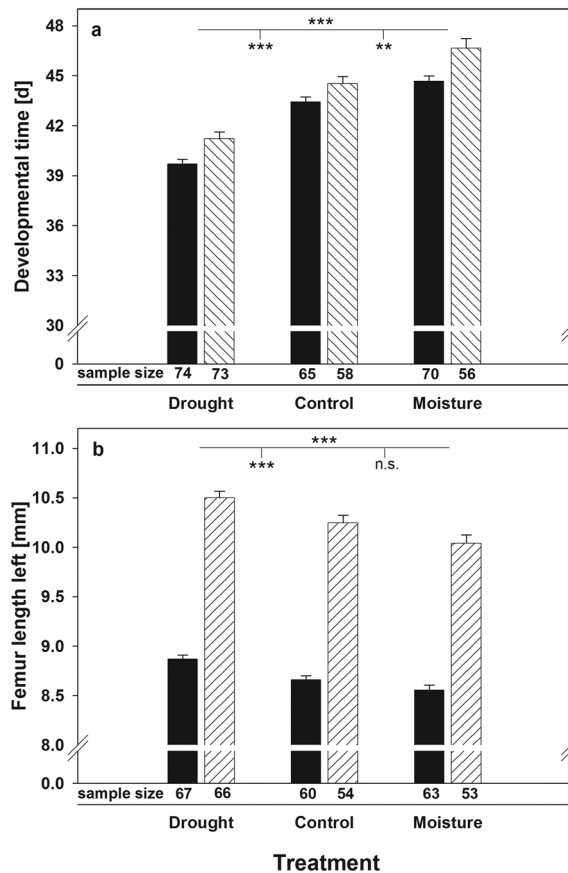

Fig. 1. Condition parameters: (a) developmental time from hatching until final molt as a function of food plant water treatment, (b) femur length of adult individuals as a function of food plant water treatment. Black bars represent males and striped bars females. Bars represent means ± SE and numbers below bars indicate sample sizes. Populations were pooled. Tukey HSD-Test: <0.001 '***', <0.01 '**', <0.05 '*', not significant 'n.s.'

Body size, body mass and condition

Body size.—Femur length of adult individuals differed significantly between insects kept on plants of the different water treatments (LME: $F_{2,27} = 28.30$, $p < 0.001$, Fig. 1b) and sexes (LME: $F_{1,328} = 1015.80$, $p < 0.001$, Fig. 1b). We found significant pair-wise differences between the treatments except control vs. moisture (Tukey HSD-Test: all pair-wise comparisons: $p < 0.001$, except control vs. moisture $p = 0.073$). Individuals of the drought treatment were on average 2.5% larger than individuals of the control

treatment and about 4% larger than individuals of the moisture treatment (Appendix: Table A1). Individuals of the control treatment were on average about 2% larger than individuals of the moisture treatment (Appendix: Table A1).

Body mass.—Body mass differed significantly between insects kept on plants of the different treatments (LME: $F_{2,27} = 11.20$, $p < 0.001$) as well as between the sexes (LME: $F_{1,364} = 793.80$, $p < 0.001$). We found significant differences between all water treatments (Tukey HSD-Test: drought vs. control $p = 0.021$ and drought vs. moisture $p = 0.003$ but not between control and moisture $p = 0.817$), while controlling for the effects body size resulted in: LME: $F_{1,327} = 256.10$, $p < 0.001$. Individuals of the drought treatment were on average about 4% heavier than individuals of the control treatment and about 6% heavier than individuals of the moisture treatment (Appendix: Table A1). Females of the control treatment were on average about 3.5% heavier than females of the moisture treatment. Male body mass showed no difference between the control and moisture group (Appendix: Table A1).

Reproduction of grasshoppers

Number of egg cases.—The number of egg cases laid per female grasshopper differed only between drought and moisture treatment, and after correction for multiple comparisons the p-value of drought vs. control was not significant (Poisson GLMM: drought vs. control $p = 0.047$ and drought vs. moisture $p = 0.012$, between control and moisture $p = 0.530$, Fig. 2a; Tukey HSD-Test: $p > 0.05$, except drought vs. moisture $p = 0.032$), while controlling for the effects of body size (Poisson GLMM: $p = 0.158$). Individuals of the drought treatment laid on average 20% more egg cases than individuals of the control treatment and about 26% more egg cases than individuals of the moisture treatment (Appendix: Table A1). Individuals of the control treatment laid about 7% more egg cases than individuals of the moisture treatment (Appendix: Table A1).

Number of eggs in the case.—The average number of eggs per egg case differed between water treatments of plants (LME: $F_{2,27} = 6.48$, $p < 0.01$; Tukey HSD-Test: drought vs. control $p = 0.903$, drought vs. moisture $p = 0.002$, control vs. moisture $p = 0.010$; Fig. 2b) while controlling for
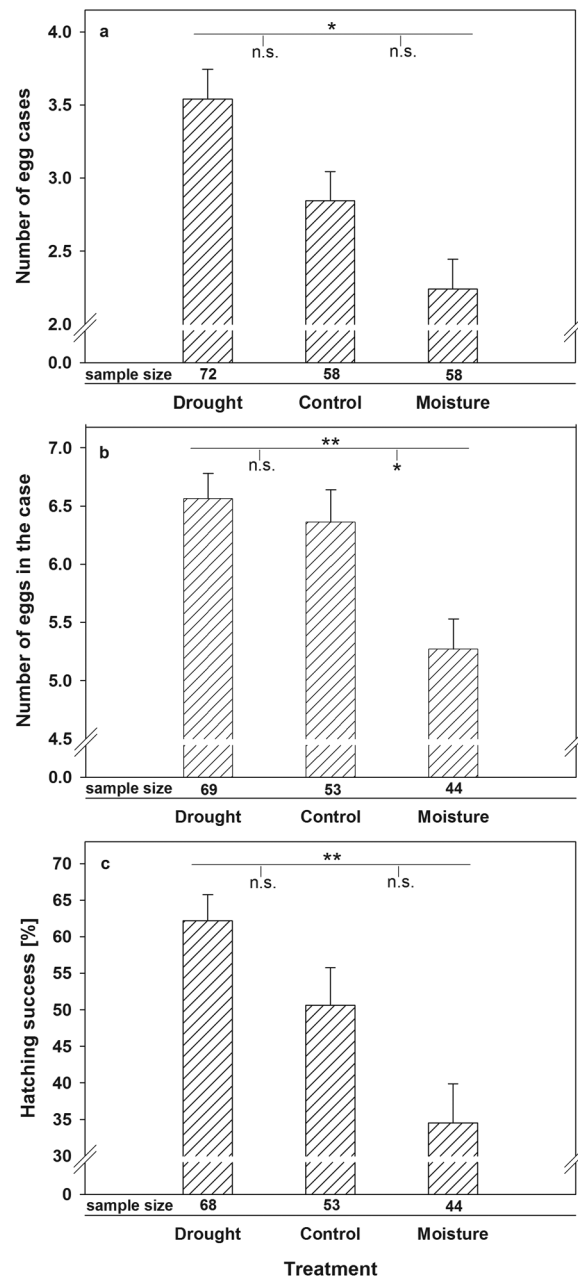

Fig. 2. Reproductive performance as a function of food plant water treatment: (a) number of egg cases per female, (b) number of eggs per egg case, (c) percentage of hatching success. Bars represent means ± SE and numbers below bars indicate sample sizes (number of females). Populations were pooled. Tukey HSD-Test: <0.001 '***', <0.01 '**', <0.05 '*', not significant 'n.s.'

the effects of body size (LME: $F_{1,118} = 5.83$, p = 0.017). Individuals of the drought treatment laid on average 3% more eggs per case than individuals of the control treatment and about 20% more eggs per case than individuals of the moisture treatment (Appendix: Table A1). Individuals of the control treatment laid about 17% more eggs per case than individuals of the moisture treatment (Appendix: Table A1).

Mass of egg cases.—The average fresh mass of an egg case differed between water treatments of plants (LME: $F_{2,27} = 11.24$, p < 0.001; Tukey HSD-Test: drought vs. control p = 0.048, drought vs. moisture p < 0.001, control vs. moisture p = 0.044) while controlling for the effects of the number of eggs in the case (LME: $F_{1,131} = 59.50$, p < 0.001) and female body size (LME: $F_{1,120} = 24.74$, p < 0.001). Individuals of the drought treatment laid egg cases which were on average 11% heavier than individuals of the control treatment and about 22% heavier egg cases than individuals of the moisture treatment (Appendix Tab. 1). Individuals of the control treatment laid egg cases about 13% heavier than individuals of the moisture treatment (Appendix: Table A1).

Offspring hatching success.—Whether offspring hatched or not was related to different water treatments of plants (Binomial GLM: drought vs. control p = 0.019 and drought vs. moisture p = 0.001 but not between control vs. moisture p = 0.149, Fig. 2c; Tukey HSD-Test: drought vs. control p = 0.049, drought vs. moisture p = 0.001, control vs. moisture p = 0.317). Hatching success (average number of offspring as related to the number of laid eggs in the cases) differed among water treatments except drought vs. control and control vs. moisture (LME: $F_{2,27} = 23.62$, p < 0.001; Tukey HSD-Test: drought vs. control p = 0.700, drought vs. moisture p = 0.006, control vs. moisture p = 0.061, Fig. 2c). Hatching success of females of the drought treatment was about 19% higher than of females of the control treatment and about 45% higher than of females of the moisture treatment (Appendix: Table A1). Hatching success of females of the control treatment was about 32% higher than of females of the moisture treatment (Appendix: Table A1).

Plant compounds

With respect to soluble protein content and the concentration of ten different amino acids we
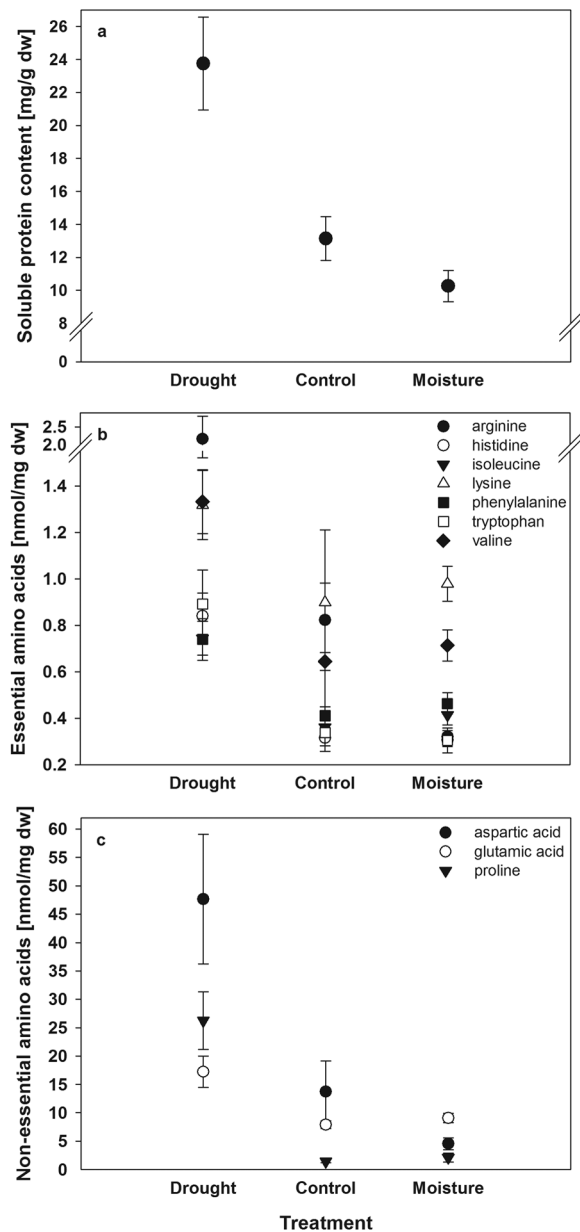

Fig. 3. Plant contents: (a) soluble protein content, (b) concentration of essential amino acids and (c) concentration of non-essential amino acids. Figures represent means of all five plant species (*Agrostis capillaris*, *Dactylis glomerata*, *Festuca rubra*, *Poa pretense*, *Trifolium repens*) over five weeks. Sample size = 15 (per week of all treatments). Stage of plants: vegetative. Symbols represent means ± SE.

found differences between the water treatments all over weeks (Fig. 3; Appendix: Table A2). The

plants of the drought treatment revealed the highest content of soluble protein, whereas the plants of the moisture treatment showed the lowest level (drought: 45% more than control, 57% more than moisture, control: 21% more than moisture; Appendix: Table A2). In ten amino acids we found highest concentrations in plants of the drought treatment and lowest concentrations in plants of the control or moisture treatment (Appendix: Table A2). Flavonoids and phenolic compounds were detected in *Trifolium repens*, but not in grass species. *T. repens* of the drought treatment had higher concentrations of secondary plant compounds than *T. repens* of the control or moisture treatment (Appendix: Table A3).

DISCUSSION

In the current study we tested whether or not the availability of water to food plants, during severe drought and moisture events, affect life-history traits including reproductive success of the herbivore *C. biguttulus*. We simulated drought and moisture events in a greenhouse by manipulating water availability to food plants. We found significant differences between the three treatment groups in all examined grasshopper life-history traits including reproductive success and in plant compounds. For all examined parameters of grasshoppers and plant compounds the largest differences existed between drought versus moisture conditions. These results suggest that *C. biguttulus* grasshoppers perform best when feeding on drought-stressed plants and worst when feeding on plants growing under moisture conditions which we propose is an effect of the observed differences in plant compounds.

Under drought conditions plants had the highest protein and amino acid content and the lowest under moisture conditions. As proteins are an important nitrogen source for insects, we assume that the increased soluble protein content and amino acid concentrations show that nitrogen is better available in plants under drought stress. Our analyses of some secondary plant compounds showed that only *Trifolium repens* had highest amounts of secondary compounds under drought stress. In grasses we found no evidence for chemical plant defenses. *T. repens*

showed higher concentrations of secondary plant compounds in plants growing under moisture conditions, like some undefined flavonoids and phenolic compounds, with the exception of cyanogenic glycosides that were increased in plants growing under drought conditions. Given that *T. repens* was the only plant species known to contain secondary plant compounds, it is possible that *C. biguttulus* is not affected by defense compounds of this plant species. Since grasshoppers are mainly feeding on grass species it is also likely that other defense compounds are more important than the examined secondary ones. Plants of the control treatment had intermediate contents in all analyzed plant compounds. It was not measured to which extent the different plant species were consumed by the grasshoppers in their respective treatment and therefore we cannot analyze whether treatment had an effect on the composition of the diet.

The better performance of grasshoppers that fed on drought-stressed plants seems to be associated with a higher soluble protein content and higher amounts of ten amino acids. Generally, for insect herbivores, available dietary N (protein and free amino acids) can potentially limit population processes like growth and dispersal (McNeil and Southwood 1978, Mattson 1980, White 1993). Consequently insect herbivores like species of the orders Orthoptera and Lepidoptera benefit from increased available nitrogen in their food plants (White 1976, Slansky and Feeny 1977). The statement that a higher nitrogen concentration in food plants often leads to faster growth rates in herbivores was postulated in a number of different studies (Traw et al. 1996, Williams et al. 1997, Hättenschwiler and Schafellner 1999). This is in accordance with the observed relationship between soluble protein content as well as amino acid concentration and body size, where individuals that fed on drought-stressed plants were larger and heavier than individuals grown under control or moisture conditions. Naturally occurring protein levels in host plants can limit grasshopper growth (Dadd 1960, 1985, Joern and Gaines 1990). In *Ageneotettix deorum* (Orthoptera, Acrididae), host plant protein concentrations were of major importance in limiting demographic parameters (e.g., fertility, mortality) (Joern and Behmer 1997). This view is also

underpinned by the finding that grasshoppers prefer to feed on plants with high amounts of amino acids like proline and valine to increase their fitness (Hsiao 1973, Haglund 1980). The observed faster development of *C. biguttulus* under drought stress could be caused by the higher availability of soluble proteins and increased amino acid concentrations of food plants as nitrogen is known to be a considerable limiting factor for herbivore development (Strong et al. 1984), including grasshoppers (McGinnis and Kasting 1966, Bernays and Chapman 1978). Haglund (1980) reported for grasshoppers and Kuhlmann and Mueller (2010) for aphids that individuals consuming proline in increased quantities grow faster. In line with this argument, proline was one of the amino acids available in significantly higher amounts in plants growing under drought conditions. Also the number of eggs in egg cases differed significantly between all treatments and was highest in females feeding on drought-stressed plants and lowest in females feeding on plants growing under moisture conditions. Besides body size that had a slight effect on the number of eggs in cases, the reason for the observed variation in egg number between the different treatment conditions could be that grasshopper ovaries consist of a series of ovarian follicles which are typically not all producing eggs under resource-limited conditions (Bellinger et al. 1987, Joern and Gaines 1990, Branson 2003a, 2003b, 2004). This would coincide with our finding that fewer eggs per case are laid under moisture conditions of host plants which is associated with lower availability of proteins and amino acids. The higher number of egg cases and accordingly the higher number of offspring of females that fed on drought-stressed plants seem to be associated with a higher suitability of plants because plant nutrients are more concentrated or better balanced (Mattson and Haack 1987). We conclude that the key for understanding differences in performance of grasshoppers between treatments are the nutrients in plants.

The frequency of drought and moisture events is predicted to increase in the future (IPCC 2007) and the effects that those stress events can have on plants are likely to influence grasshopper performance. Drought stress condition of plants can be expected to lead to better performance of *C. biguttulus* grasshoppers, and worse performance is expected under severe moisture conditions of food plants.

We conclude that different water regimes of food plants may have different effects on grasshopper performance. We suggest that these effects are due to changes in amounts of soluble protein and amino acids of plants under drought and moisture conditions. It should be investigated in future studies if the observed effects of plant stress on the grasshoppers via grasshopper-plant interactions might have significant long-term impacts on herbivore pressure, community dynamics and ecosystem stability. With our study we can state that grasshoppers feeding on plants growing under severe moisture conditions will suffer with regard to life-history traits including fecundity, in contrast to grasshoppers feeding on plants growing under drought conditions that show strong benefits.

ACKNOWLEDGMENTS

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APPENDIX

Table A1. Performance parameters of grasshoppers in the three treatment groups (drought, control, moisture). Populations were pooled. Values are means ± SE with N in parentheses; N = number of males/females.

Variable	Drought		Control		Moisture	
	Male	Female	Male	Female	Male	Female
Developmental time [days]						
P1	39.96 ± 0.50 (26)	42.51 ± 0.58 (35)	42.84 ± 0.41 (37)	44.85 ± 0.83 (26)	45.03 ± 0.45 (31)	47.65 ± 0.82 (31)
P2	39.56 ± 0.31 (48)	40.03 ± 0.49 (38)	44.21 ± 0.38 (28)	44.35 ± 0.37 (34)	44.38 ± 0.45 (39)	45.41 ± 0.64 (27)
Femur length [mm]	8.87 ± 0.04 (67)	10.50 ± 0.07 (66)	8.66 ± 0.04 (60)	10.24 ± 0.08 (54)	8.55 ± 0.05 (63)	10.04 ± 0.08 (53)
Body mass [mg]	72.98 ± 0.85 (74)	106.89 ± 1.91 (73)	69.67 ± 0.87 (65)	103.63 ± 2.09 (60)	70.13 ± 0.94 (70)	99.97 ± 1.95 (58)
No. egg cases		3.54 ± 0.20 (72)		2.84 ± 0.20 (55)		2.63 ± 0.21 (46)
No. eggs in the case		6.56 ± 0.22 (69)		6.36 ± 0.28 (53)		5.27 ± 0.26 (44)
Mass of egg cases [mg]		72.51 ± 1.71 (69)		64.70 ± 1.82 (55)		56.35 ± 2.48 (46)
Hatching success [%]		62.20 ± 3.60 (69)		50.70 ± 5.10 (53)		34.50 ± 5.40 (44)

Table A2. Plant contents and concentrations (soluble protein, essential and non-essential amino acids) of a combination of five food plant species (*Agrostis capillaris, Dactylis glomerata, Festuca rubra, Poa pretense, Trifolium repens*) exposed to three treatment groups (drought, control, moisture) over five weeks. Sample size = 15 (per week of all treatments). Stage of plants: vegetative.

Variable	Treatment $F_{2,71}$	Treatment p	Week $F_{1,71}$	Week p	Drought vs. Control p	Drought vs. Moisture p	Control vs. Moisture p	Drought Mean ± SE	Control Mean ± SE	Moisture Mean ± SE
Soluble protein content [mg/g]	14.27	<0.001	1.05	0.310	0.001	<0.001	0.529	23.75 ± 2.82	13.14 ± 1.33	10.26 ± 0.95
Amino acids [nmol/mg]										
Arginine	17.35	<0.001	1.54	0.219	<0.001	<0.001	0.685	2.16 ± 0.64	0.82 ± 0.39	0.32 ± 0.03
Asparagine	18.08	<0.001	4.01	0.049	<0.001	<0.001	0.616	47.67 ± 11.44	13.72 ± 5.41	4.54 ± 1.08
Glutamine	7.76	0.001	0.28	0.600	0.001	0.012	0.716	17.23 ± 2.77	7.88 ± 0.68	9.11 ± 0.82
Histidine	21.89	<0.001	0.44	0.509	<0.001	<0.001	0.998	0.84 ± 0.10	0.32 ± 0.03	0.31 ± 0.03
Isoleucine	13.06	<0.001	3.40	0.070	<0.001	<0.001	0.812	0.74 ± 0.07	0.36 ± 0.03	0.41 ± 0.04
Lysine	4.37	0.016	2.52	0.117	0.019	0.069	0.856	1.32 ± 0.15	0.90 ± 0.08	0.98 ± 0.08
Phenylalanine	8.09	0.001	2.27	0.137	0.001	0.007	0.817	0.74 ± 0.09	0.41 ± 0.04	0.46 ± 0.05
Proline	43.53	<0.001	0.08	0.779	<0.001	<0.001	0.947	26.24 ± 5.28	1.45 ± 0.27	2.11 ± 0.76
Tryptophane	12.30	<0.001	0.18	0.677	<0.001	<0.001	0.962	0.89 ± 0.15	0.34 ± 0.08	0.30 ± 0.05
Valine	18.59	<0.001	5.98	0.017	<0.001	<0.001	0.875	1.33 ± 0.14	0.64 ± 0.04	0.71 ± 0.07

Table A3. Ratio of undefined secondary plant compounds of *Trifolium repens* of the three plant treatment groups (drought, control, moisture).

Compound	Drought vs. Control	Drought vs. Moisture	Control vs. Moisture
Flavonoid	1:1.2	1:2.3	1:1.9
Phenolic compound 1	1:2.2	1:4.8	1:2.2
Phenolic compound 2	1:1.6	1:2.8	1:1.8
Phenolic compound 3	1:1.4	1:8.4	1:6.2
Phenolic compound 4	1:1.2	1:2.9	1:2.4