Effect of Salinity and *Bacillus subtilis* on White Fly (*Trialeurodes vaporariorum*, Westwood) in Hydroponically Grown Tomatoes (*Lycopersicon esculentum* Mill.)

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Keywords: *Bacillus subtilis*, tomato, *Lycopersicon esculentum* Mill., white fly, *Trialeurodes vaporariorum* Westwood, insect population development

Abstract

Motivation for this investigation was the recognition that plant physiological responses will change under different salt levels in the nutrient solution with potential impact on white fly behavior. *Bacillus subtilis* induces plant resistance to stress and produces plant hormones for growth improvement. White fly population growth and development were examined on tomato plants grown under 3 salinity regimes (EC 0, 2, 4 NaCl) in an environment controlled greenhouse. In addition, the high Na-salinity level (EC 7.4) was inoculated with the plant growth promoting bacteria *B. subtilis* in the root zone. White fly immature counts had distinct trends with the largest numbers occuring in the highest salinity level with *B. subtilis*. However, this treatment produced the most vigorous vegetative growth with significantly larger leaf size as compared to the other treatments. White fly population dynamics are discussed in relation to sugar concentrations (glucose, fructose, sucrose, oligosaccharides and polysaccharides) as well as N- and NaCl-contents in the tomato leaves of plants grown under the 4 salinity regimes.

INTRODUCTION

Salinity induces many stresses to plants such as osmotic stress, ionic imbalances (Epstein and Rains, 1987, Läuchli and Epstein 1990) and toxic effects (Hasegawa et al., 2000). They react manifold to rising salintiy in the nutrient solution by physiological adaptations, adjusting the osmotic potential of the cells by an increase in compatible solutes (McNeil et al., 1999, Rhodes et al. 2002) or by compartimentation (Blumwald, 2000, Niu et al., 1995).

If salinity rises to a certain species dependent threshold, a reduction in growth and fruit production due to reduced water uptake (Bano *et al.*,1993), energy loss (Stavarek and Rains, 1995), and disordering effects (on the physiological, membrane or molecular level) emerges (Pitman and Läuchli, 2002).

Abiotically (salinity) stressed plants have demonstrated to be more susceptible to herbivore attacks and feeding (Schoenweiss, 1975, Jarvis, 1992, Engelhard, 1989, Heil 1999), but it could be also conceivable that certain forms of stress hardens the plants against another stress and/or indirectly, the stressed plants remain less palatable or are better defended after the stress occurred (Grierson et al., 1982). For instance, it is reported from *Bemisia argentifolii* feeding on water stressed plants that population development is slowed down even though the diet in sugars is richer, due to higher concentrations (Salvucci et al., 1997), a stress effect which is similar to salinity stress. The nitrogen (N) status (aminoacids) of the plant plays a crucial role for insect feeding and population growth (Crafts-Brandner, 2002), but even N uptake is influenced by the the presence of chloride (Grattan et al., 1992, Caron et al., 1991).

Salinity is a widespread problem in crop cultivation, therefore we focused on plant reaction on it, but we were also interested to look at the indirect effects of salinity induced stress with respect to the feeding insect pest under these conditions, addressing the findings to possible IPM strategies.

MATERIALS AND METHODS

Plant Material and Culture Conditions

Tomatoes (cultivar Douglas, Fa Juliwa, Germany) were grown in a closed soilless hydroponical system from March 21 (transplanting) – July 21, 2004, in a climatically controlled greenhouse (320 m^2, Freising, Germany). Perlite was used as subtrate (0-6 mm) in 10 l pots. Plant density was 2 plants/pot, 3 plants/m^2 (0.3 x 1.2 m), 30 plants/treatment.

Salinity levels, fertigation and watering: EC 3.4 (nutrient solution plus 5mM $CaCl_2$ without NaCl (control)); EC 5.4 (nutrient solution plus 5mM $CaCl_2$ plus 17mM NaCl), EC 7.4 (nutrient solution plus 5mM $CaCl_2$ plus 34 mM NaCl), EC 7.4 (nutrient solution plus 5mM plus 34 mM NaC)l plus *Bacillus subtilis*. Salinization of the nutrient solution started 20th of April without any plant adaptation.

Nutrient solution: Macronutrients (mmol) 10 NO_3, 1 NH_4, 6.5 K, 1.25 P, 1.25 Mg, 1.5 S, 1 Cl. Micronutrients (µmol) 15 Fe, 10 Mn, 20 B, 0.75 Cu, 4 Zn, 0.5 Mo.
Every two weeks nutrient solutions were analyzed for adjustments of the stock solution to guarantee an optimal nutrient supply to the plants (Programm Substrafeed, Fa Hydro Agri, The Netherlands). Sodium chloride and calcium chloride in the nutrient solution were adjusted 2-times a week and, if necessary, adpated to requested levels (Test kids, Fa Merk, Germany). Watering (drip irrigation, 4 drippers per pod) was at fixed intervals, every 20 min for 5 min (8l /h) in a closed system.

Bacillus subtilis strain FZB24®WG (FZB, Biotechnik GmbH, Germany) inoculation took place at the 4 leaves stage of the juvenile plants and was applied as spore solution (0,02% w/w) 7 times over a week (ones each day, ~50 ml/plant). Additionally, after transplantation into the greenhouse, the spore solution was added one more time.

White fly (*Trialeurodes vaporariorum* Westwood) was introduced as pupae in a caged part (100 m^2) of the greenhouse. On 21st of May, 5 plants per treatment were infested with 50 pupae shortly before hedging. Therefore, infested leaves with 50 pupae were attached to the sixth leaf from the top of the plants. A second infestation was undertaken on 27th of June, in contrast to the first application, 100 adults were released per plant.

White Fly Counts

White fly numbers (eggs, larvae and pupae) were counted in certain time intervals (13.6./ 15.7. - whole plant, 21.7. – from 3 leaf discs/plant (2,53 cm^2 each) on the youngest fully developed leaves.

Evaluation Parameters

From the youngest fully developed leaves July 21 sugar analyse were made for glucose, sucrose and fructose (n = 7). Element analyze were conducted from the youngest fully expanded leaves collected on April 8, May 8 and July 15 (C, N, Ca, Mg, K, Na and P).

Statistical Analysis

Statistical analysis was conducted using Statistica (Version 5.5, Tulsa, USA, 1999). Comparing whitefly development in relation to salinity a Kruskal-Wallis-Anova was applied, pairwise comparisons were made by Mann-Whitney U-Test. Statistical analysis of sugars based on analysis of variance (F-Test), Least Significant Difference (LSD) were used for the comparison between treatments. Values (means, medians) are supposed to be significantly different at the 5 % probability level.

RESULTS AND DISCUSSION

White fly population development in terms of larvae and pupae counted at whole plant level on July 15th tended in higher numbers of pupae in the salinity treatments, whereas larvae had highest counts in the control treatment. The sum of both life stages were the highest in the *Bacillus* treatment. This development could be interpreted as a faster insect stage development under saline conditions (Fig. 1A).

White fly numbers all three stages (eggs, larvae, pupae) on youngest fully developed leaves had a significant relationsship between salinity and numbers of white fly larvae as well as the the sum of all life stages counted (Fig. 1B). Comparable to the survey made on 15th July, the population development appeared to be faster in terms of higher numbers of advanced life stages under higher salinity levels. Again the *B. subtilis* inoculated plants yielded the highest numbers of larvae and even the sum out of all three stages together, even though pairwise comparisons between high salinity treatments, with and without *B. subtilis* inoculated plants, were at no time significantly different. Reviewing these results affirm the hypothesis that stressed plants are more prone and susceptible to pests and diseases (Schoenweiss, 1975, Berlinger et al., 1999).

We expected further confirmation for this development by the analysis of leaf tissue sugar content (glucose, fructose, sucrose) and nutrient element concentrations, especially N, known that both are most important constituents for the the growth and development of plant feeding insects (Scriber, 1984, Crafts-Brandner 2002, Salvucci et al., 1997).

Total sugar contents of mature leaf tissue had no significant differences among salinity levels per se (Fig. 2). Only the highest salinity treatment with *B. subtilis* was clearly distinguishable, for the pattern and the amount of sugars. The *B. subtilis* variant had significantly lowered amounts of fructose and smallest amounts of the sum of glucose, fructose and sucrose as dry weight (sucrose and glucose were not significantly different). If fructose or the proportion of single sugars played a role for the tendency of white fly population development, then this could explain for the faster insect life cycle in the *B. subtilis* treatment (Fig. 1B, Salvucci et al., 1997).

Nutrient element concentrations also had remarkable differences in relation to salinity levels during the time course of the experiment as well as in relation to the *B. subtilis* treatment (Mg, K, Ca). The already mentioned importance of N-concentrations in the leaf tissue were most obviously influenced by *B. subtilis* and yielded the highest concentrations over the whole growing period, looking at newly grown tissue (weekly pruned side shoots) as well as mature leaf tissue (Table 1), with a tendency of decreasing differences towards later developed leaves in the growing season. This may be due to a dwindling activity (population decrease) of *B. subtilis* on the roots which is reported and decribed for this organism (Batinic et al., 1998, Grosch et al., 1996). Also the C/N-ratios in mature leaf tisssue were more advantagous in the *B. subtilis* plants, shifted to higher N and lower C-concentrations as compared to the other treatments (Fig. 3). Other differences observed, such as vegetative plant growth with possible consequences for the development of white fly (Berlinger et al., 1999) are summarized more detailed in Woitke et al. (2004), here to note only leaf area and numbers which were significantly influenced by the *Bacillus* treatment.

Summarizing these preliminary results on increasing salinity levels could be seen as a factor favouring white fly development and numbers on tomato plants, in this regard with no positive implications directly for IPM. Albeit direct measurement of leaf sugar content and leaf nutrient element concentrations onto white fly population development, no direct conclusion can be made, because the feeding behaviour of the sucking insect will require the analysis of the phloem sap itself, taken the tendency into consideration that *B. subtilis* inoculated plants had always the highest numbers of white flies. This implies that the microorganism does improve and change the physiological properties of the plant with direct relevance to the sucking insect.

ACKNOWLEDGEMENTS

This work is funded and supported by the European Union under the INCO-MED2 ECOPONICS project (FP5RTD), Contract N° ICA3-CT-2002-10020.

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Tables

Table 1. Nutrient element concentrations (g/100g dw) in youngest fully developed leaves before salinization, 20 d after and at the end of the cultivation period (compounded samples n = 30).

	Before salinization (April 8)				20 d after salinization (May 8)				90 d after salinization (July 15)			
	C	5.4	7.4	7.4 + B.s.	C	5.4	7.4	7.4 + B.s.	C	5.4	7.4	7.4 + B.s.
Ca	2.20	2.45	1.70	2.12	2.05	2.19	1.78	2.64	3.72	4.04	4.01	4.78
Mg	1.16	1.21	0.91	1.03	0.45	0.42	0.37	0.53	0.66	0.57	0.80	0.93
K	4.57	5.16	4.27	5.01	5.64	5.60	5.48	5.41	4.52	4.00	3.72	3.41
P	0.63	0.75	0.59	0.67	0.86	0.83	0.98	0.70	0.64	0.66	0.57	0.64
C	37.4	36.2	38.6	37.5	38.0	38.2	38.1	34.3	34.4	35.9	35.5	34.5
N	4.41	4.91	4.43	4.54	4.59	4.95	4.54	5.79	4.48	4.03	4.44	5.79
Na	0.11	0.14	0.10	0.12	0.05	0.42	0.37	1.50	0.08	0.65	0.87	1.00

Figures

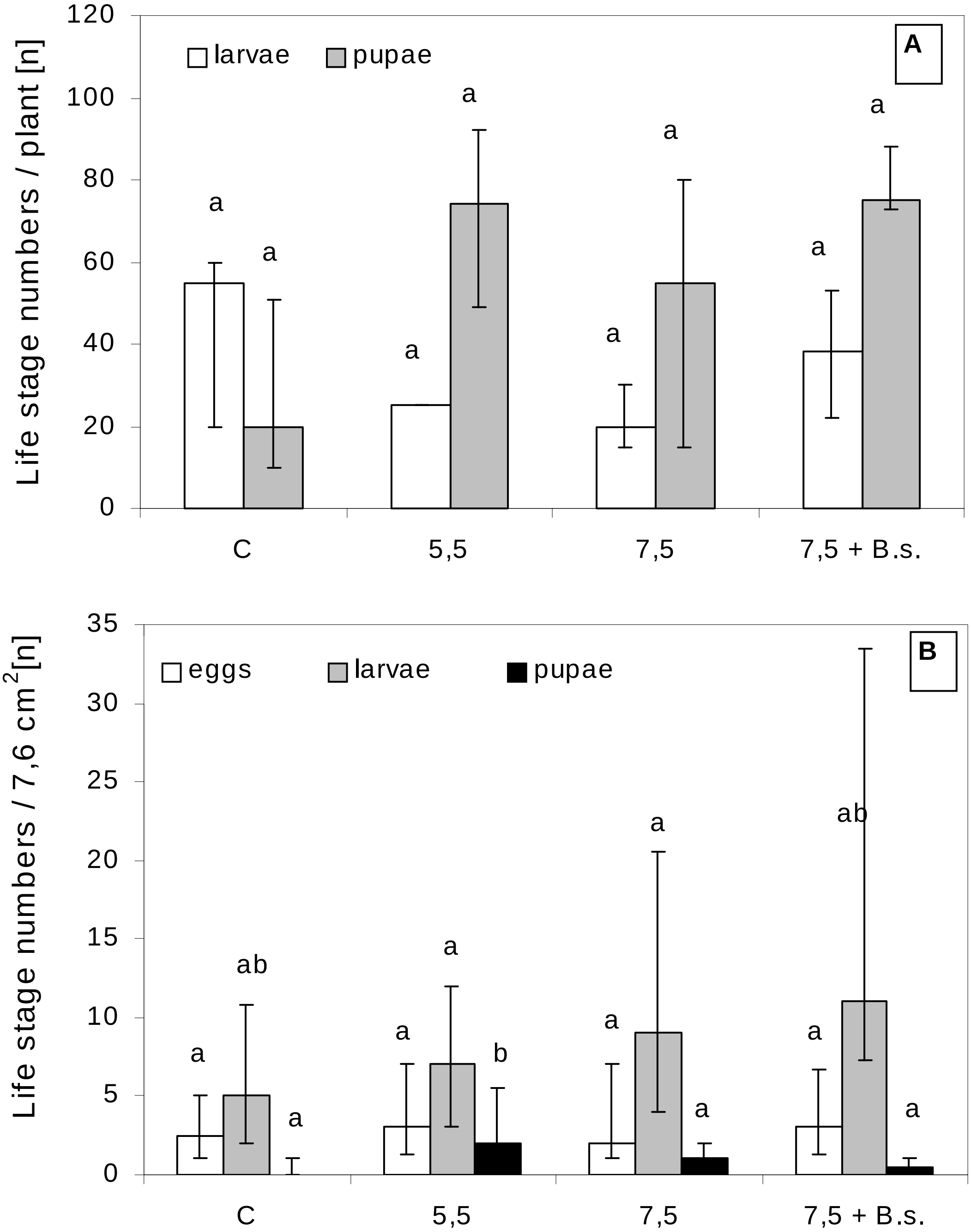

Fig.1. Larvae & Pupae counts (July 15, A and July 21 B, 2003) in relation to salinity and *B. subtilis* treatment. Countings in Fig. A refer to the whole plant, in Fig. B to the youngest fully developed leaf. Columns indicate medians n = 5, error bars quartils (25-75%), statistics, Mann-Whitney U-Test < 0.05.

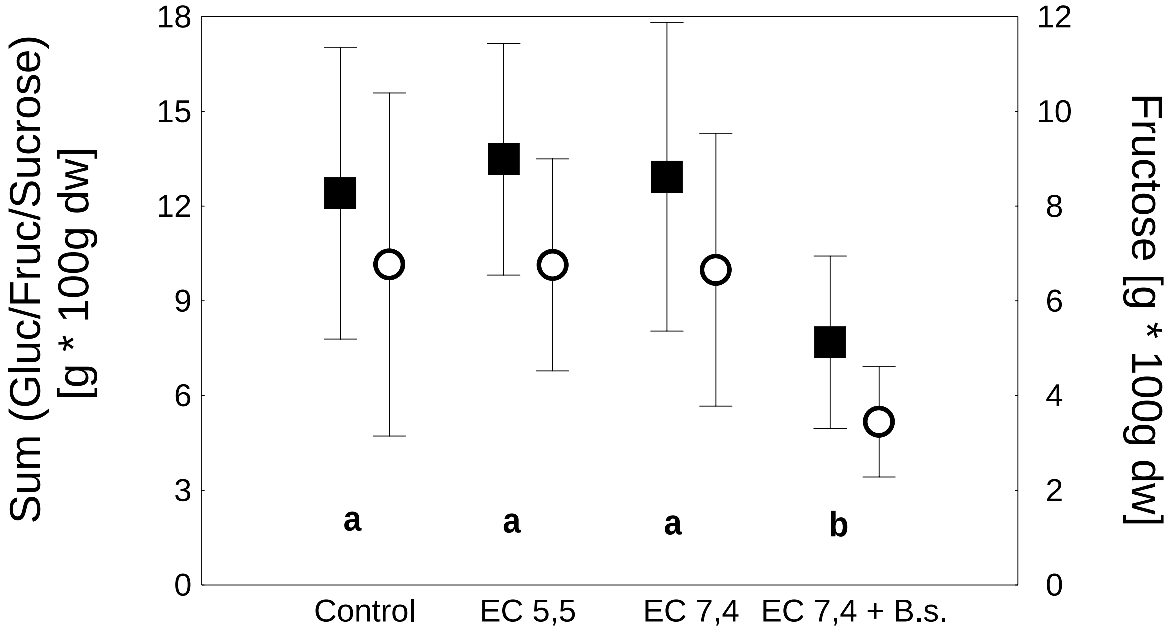

Fig. 2. Sugar concentrations (closed sysmbols - sum of glucose, fructose, sucrose; open symbols – fructose only) in mature leaf tissues, when countings of white fly were made 21st of July, 2003 in relation to salinity levels and *B. subtilis* inoculation (n = 7, symbols indicate means, error bars ± s.d.).

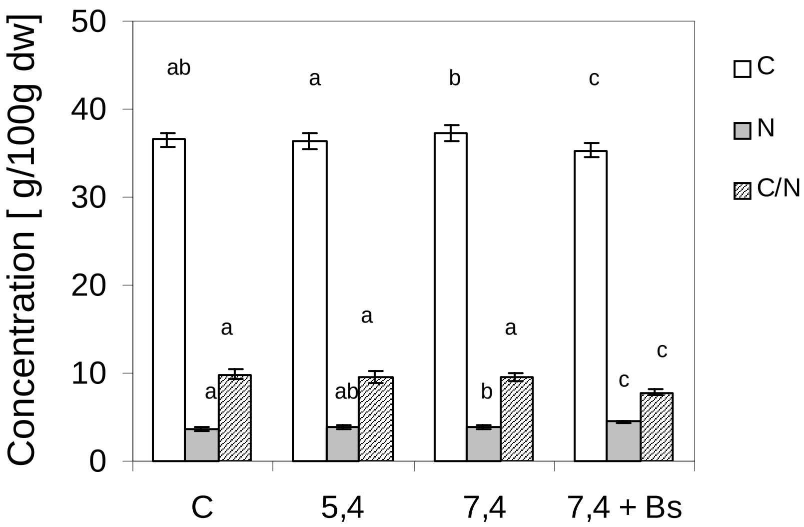

Fig. 3. C-, N concentrations and C/N-ratios of mature leaf tissues (June, 24th) in relation to salinity levels and *B. subtilis* inoculation n = 10, columns indicate means, error bars ± s.d.).