J Chem Ecol (2016) 42:1293–1303
DOI 10.1007/s10886-016-0795-7

Does Plant Cultivar Difference Modify the Bottom-Up Effects of Resource Limitation on Plant-Insect Herbivore Interactions?

Peng Han[1,2] · **Nicolas Desneux**[1] · **Thomas Michel**[3] · **Jacques Le Bot**[4] · **Aurelie Seassau**[1] · **Eric Wajnberg**[1] · **Edwige Amiens-Desneux**[1] · **Anne-Violette Lavoir**[1]

Received: 14 February 2016 / Revised: 30 October 2016 / Accepted: 1 November 2016 / Published online: 26 November 2016

Abstract Variation in resource input to plants triggers bottom-up effects on plant-insect herbivore interactions. However, variation in plant intrinsic traits in response to resource availability may modify the bottom-up effects. Furthermore, the consequences also may depend on the feeding strategy of insect herbivores belonging to different feeding guilds. We evaluated the performance of two insect herbivores from distinct feeding guilds, the leaf miner *Tuta absoluta* and the phloem feeder *Bemisia tabaci*. We offered the insects two tomato cultivars growing under optimal nitrogen input vs. nitrogen limitation, or under optimal water input vs. water limitation. We found that: (i) the two cultivars differed in their responses to nitrogen and water limitation by regulating primary (leaf-gas exchange related parameters, leaf nitrogen content, and leaf C/N ratio) and secondary metabolism (main defensive compounds: glycoalkaloids); (ii) for both plant cultivars, nitrogen or water limitation significantly affected *T. absoluta* survival and development, while *B. tabaci* survival was affected only by nitrogen limitation; and surprisingly (iii) plant cultivar differences did not modify the negative bottom-up effects of resource limitation on the two insect herbivores. In conclusion, the negative effects of resource limitation cascaded up to insect herbivores even though plant cultivars exhibited various adaptive traits to resource limitation.

Keywords Nitrogen · Water · Glycoalkaloid · *Solanum lycopersicum* · *Tuta absoluta* · *Bemisia tabaci*

Introduction

Plant-arthropod interactions are thought to be of utmost importance for understanding the dynamics of ecological communities (Sarmento et al. 2011). The quality/quantity of resources available for plants triggers bottom-up effects on plant-insect herbivore interactions, i.e., plants are affected by environmental factors, which in turn influence the performance of insect herbivores (Chen et al. 2010; Costamagna and Landis 2006; Denno et al. 2002; Han et al. 2014, 2015a, 2015b). However, the bottom-up effects are highly variable and may depend on both biotic and abiotic factors, such as plant genotype (Ballhorn et al. 2011), insect feeding strategy (Inbar et al. 2001), insect feeding specialization (Gutbrodt et al. 2011), resource type (Inbar et al. 2001), and environmental stress intensity (Mody et al. 2009).

Plant nitrogen (N) availability is a key factor influencing the performance of insect herbivores (Mattson 1980). However, no consensus has been reached on the bottom-up effects of N input on insect herbivores. The *nitrogen limitation hypothesis* (White 1993) predicts that reduced nitrogen availability to plants may impair the performance of Lepidoptera insect herbivores. The hypothesis has been supported by numerous studies (Cornelissen and Stiling 2009; Han et al. 2014;

Electronic supplementary material The online version of this article (doi:10.1007/s10886-016-0795-7) contains supplementary material, which is available to authorized users.

✉ Peng Han
penghan1394@gmail.com

[1] INRA (French National Institute for Agricultural Research) Univ. Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 400 route des chappes, 06903 Sophia-Antipolis, France

[2] College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China

[3] Institut de Chimie de Nice, UMR CNRS 7272, Université Nice Sophia-Antipolis, Parc Valrose, F-06108 Nice, France

[4] UMR1115 PSH (Plantes et Systèmes de culture Horticoles), INRA, F-84000 Avignon, France

Hunter and McNeil 1997; Inbar et al. 2001; White 1993). However, other studies have demonstrated negative effects of high plant leaf N content on insect herbivores, and thus have undermined the generality of this hypothesis (Fischer and Fiedler 2000; Joern and Behmer 1998). For several phloem-feeding insects, an increase in N input and/or optimal plant nitrogen status has been reported to enhance survival and development (Bentz et al. 1995; Bi et al. 2003; Chau et al. 2005; Hogendorp et al. 2006), perhaps because the typical nitrogen concentration of phloem sap is relatively low (Mattson 1980).

Water is another crucial resource for plants and insect herbivores. Varying water availability may induce physiological changes in terms of plant nutritional value and chemical defenses, thus affecting insect herbivores (Gutbrodt et al. 2011; Tariq et al. 2012). Insects from different feeding guilds, e.g., chewing or sap-sucking, may be influenced differently by water limitation. However, there is no conclusive literature on this subject since positive, negative, or even non-significant responses have all been documented for chewers and sap feeders (Huberty and Denno 2004). Both the *plant vigor hypothesis* (Price 1991) and the *plant stress hypothesis* (White 1993) have been put forward to explain the mixed results. The former predicts that insect herbivores perform better on rapidly-growing vigorous plants (Price 1991), and this has been supported particularly for leaf miners and leaf chewers (Han et al. 2014; Inbar et al. 2001). The latter predicts that water-stressed plants are more suitable hosts for senescence-feeders that prefer to consume over-mature and senescing tissues (White 2009). Finally, drought patterns also can influence insect herbivores. The *pulsed plant stress hypothesis* has been proposed to explain discrepancies between sap feeder outbreaks observed *in situ* on water-stressed plants and the negative effects on phloem feeders detected in laboratory studies (Huberty and Denno 2004). The hypothesis predicts that phloem feeder populations, e.g., aphids or whiteflies, respond positively to plants enduring discontinuous stress because bouts of stress followed by recovery of turgor allow phloem feeders to benefit from stress-induced increases in plant soluble nitrogen. However, phloem feeders may be affected negatively by continuous water stress where the turgor pressure declines below the threshold required for efficient feeding.

Analyzing the effects of the environment on plant-insect interactions often is complicated by the diversity of plant responses to different abiotic factors (Ballhorn et al. 2011). In modern agriculture, plant cultivars that are designated as "resistant" have been selected based on their response to sub-optimal growing conditions, such as nitrogen deficiency (Feng et al. 2010) or drought (Cattivelli et al. 2008; Farooq et al. 2009). Among resistant cultivars, the impact of inter-cultivar variation on plant-insect interactions is unknown (Ballhorn et al. 2011).

The present study explores the effects of two abiotic factors, nitrogen and water inputs, on plant nutritional and defensive profiles at the cultivar level. It further examines the subsequent bottom-up effects on the performance of insect herbivores from two distinct feeding guilds. Three questions have been addressed: (i) Do different plant cultivars have different allocation patterns (in terms of changes in primary and/or secondary metabolism) when they are confronted with N and water limitations? (ii) Do insect species from distinct feeding guilds respond differently to the bottom-up effects of varying resource availability? (iii) Do different cultivars have different bottom-up effects on the insect herbivores? Our goal was to see whether the effects of resource input can cascade up to insect herbivores even when the cultivars exhibit different responses to resource limitation.

Methods and Materials

Study Organisms The biological system "tomato plant-insect herbivores" was set up under controlled laboratory conditions. We chose two different tomato (*Solanum lycopersicum* L. Solanaceae) cultivars. The cultivar "Marmande", hereafter referred to as cv. M, is commonly used under greenhouse conditions in France and is a plant model often used in plant-insect interaction studies (Chailleux et al. 2012; Han et al. 2014, 2015a, 2015b; Mouttet et al. 2013). Specifically, this cultivar is susceptible to drought conditions (Han et al. 2014). The cultivar "Noire de Crimée", hereafter referred to as cv. NC, also is used in local organic farming in France, and is commonly sold as a drought-tolerant cultivar. Both tomato cultivars were grown from seeds in a climatic chamber (24 ± 1°C, 65 ± 5% RH, 12 h light) in plastic pots (7 × 7 × 6.5 cm). After germination, seedlings were grown under laboratory conditions (25 ± 2°C, 65 ± 5% RH, 16 h light). Eight days after sowing (DAS), seedlings were washed to remove soil particles from the roots, and transferred to new cubic pots containing limestone grains (Perlite Italiana srl, Corsico, Italy) (Supplementary material 1: Fig. S1). Twenty-four DAS, plants were transferred to larger pots (diam: 10 cm, height: 9 cm) filled with the same substrate.

We used two insect species from two different feeding guilds for the study, a leaf-mining species *Tuta absoluta* (tomato leaf miner) and a phloem-feeding species *Bemisia tabaci* (silverleaf whitefly). The *T. absoluta* colony was maintained in climate chambers on tomato plants (25 ± 2°C, RH 70 ± 10%, 16 h light). Honey and water were provided *ad libitum* to adults in rearing cages. Newly-oviposited *T. absoluta* eggs were used to infest the plants. To obtain eggs, we used the method described by Chailleux et al. (2013) with ten couples of *T. absoluta* adults placed inside a plastic tube containing a fresh tomato leaf to enable mating and oviposition. The *B. tabaci* colony was maintained in chambers on

young tobacco plants (25 ± 2°C, RH 70 ± 10%, 16 h light). Two tobacco plants were put inside the cage to obtain first-instar nymphs (crawlers) that were used in the experimental tomato plant infestation.

Nitrogen and Water Inputs A full-factorial design was used to combine the two levels of nitrogen input (ON = optimal nitrogen and LN = limited nitrogen), and two levels of water input (OW = optimal water and LW = limited water). Treatments started 8 DAS and ended 60 DAS (Supplementary material 1: Fig. S1). Twenty-four plants (replicates) of each cultivar were used for each treatment combination.

Following the protocols previously described (Han et al. 2014), we used three stock solutions to dispense independently the major mineral species to the plants. The nitrate stock solution combined 0.4 M [KNO_3], 0.2 M [$Ca(NO_3)_2$], and 0.1 M [$Mg(NO_3)_2$]. The phosphate stock solution contained 0.21 M [KH_2PO_4], and the sulfate stock solution comprised 0.022 M [K_2SO_4], 0.011 M [$CaSO_4$], and 0.022 M [$MgSO_4$]. For the sulfate stock, micronutrients were provided as Kanieltra 6 Fe (Hydro Azote, Nanterre, France), and iron also was supplied as EDTA-Fe in order to obtain the following concentrations: Mo 20 μM; Mn 815 μM; Zn 227 μM; Cu 33 μM; B 1444 μM; Fe 3760 μM. On a daily basis, we supplied exponentially increasing volumes of the three stock solutions to the plants with irrigation water, following the principles described below. This nutritional regime also was used in our previous studies (Han et al. 2015a, 2015b).

Throughout the experiment, the ON treatment received a daily dose v_n (in ml) of nitrate stock solution that matched the potential plant demand for nitrogen. This was determined previously as the daily amount of N necessary to maintain plant N concentration constant during the vegetative growth period where the relative growth rate (RGR) was almost 0.1 g per g per day, as was the case in our study. During this period, v_n increased exponentially (i.e., from 0.1 ml up to 4 ml) in accordance with the RGR. To differentiate nitrogen inputs, the LN plants received only $v_n/5$ of nitrate stock solution (Supplementary material 1: Fig. S2). For each plant of both ON and LN treatments, however, the daily input volumes of phosphate and sulfate stock solutions were identical, being calculated from v_n as follows: we supplied v_n ml of phosphate and $3v_n$ ml of sulfate stocks, respectively. This method ensured that only nitrogen availability differed in the fertilization protocol. The practical application of these doses was to add the various stock solutions to the water intake (see below) of each pot in accordance with its respective water supply.

A "step increase" pattern was used for daily water inputs (Supplementary material 1: Fig. S2B). In the OW treatment, the volume (v_w) of daily water input was determined empirically as the amount required to fully-saturate the perlite substrate without visible drainage. Based on v_w, the restricted volume $v_w/3$ was applied daily per plant to set the LW treatment. All nutrient solutions were adjusted to pH 5.5 using H_2SO_4 (0.2 M).

Experimental Setup Analyses of primary and secondary plant metabolism were performed on plants before actual infestation, i.e., the *T. absoluta* larvae chewing and *B. tabaci* nymphs feeding. The goal was to examine the impact of nitrogen and water inputs on plants and to highlight the potential differences in their responses to input constraints between the two cultivars. Then, the biological traits of *T. absoluta* and *B. tabaci* were evaluated on the two plant cultivars treated with contrasting levels of nitrogen and water inputs. The terminal leaflet of the 4th fully developed leaf from the apex was used for the leaf gas-exchange measurement 38 DAS. These leaflets then were sampled 43 DAS and immediately dried at 60°C for 72 h to quantify leaf nitrogen, carbon, and glycoalkaloid content. Twenty-four plants per cultivar per treatment were sampled for plant chemical analyses, and half of the plants were selected randomly for leaf gas-exchange measurement.

Leaf Gas-Exchange Measurement Measurements of photosynthesis rate, transpiration rate, and stomatal conductance were performed. One leaflet per plant was analyzed using a portable photosynthesis system (Li-6400, Li-Cor, Lincoln, NE, USA) equipped with a light source (6200-02B LED, Li-Cor). Leaf gas-exchange measurement using Li-Cor is a non-destructive method. Leaflets first were acclimatized in the chamber for more than 20 min under controlled conditions: leaf temperature of 27.0 ± (SE) 0.1 C, CO_2 air concentration of 399.71 ± 0.04 μmol mol^{-1}, and saturating photosynthetic photon flux density (PPFD) of 700.04 ± 0.05 μmol hν m^{-2} s^{-1}.

Leaf Nitrogen Content and C/N Ratio Quantification To quantify both nitrogen and carbon content (in mg) per 100 mg of dry mass, dried leaf samples were ground into fine powder with a ball mill MM301 (Retsch, Germany) and stored individually in small tubes. Five mg of leaf dry powder were used to measure leaf nitrogen and carbon content with an elemental analyzer (Flash EA1112 Series, ThermoFinnigan, Milan, Italy) at INRA-PSH Avignon, France. The leaf C/N ratio was calculated as the ratio between the amount of carbon and nitrogen.

Leaf Glycoalkaloid Analyses Glycoalkaloids were extracted from 5 mg leaf dry powder with 2 ml of 5% acetic acid in water (*v*/v). The suspension was first mixed with a vortex and then extracted twice for 30 min using an ultrasonic assisted extractor at room temperature. After extraction, the supernatant was filtered through a 0.45 μm PVDF PuradiscTM filter (Whatman, GE Healthcare). All samples were kept at −20°C until analysis. Glycoalkaloid standards (α-tomatine and tomatidine; Extrasynthese, Genay, France) were diluted in 5% acetic acid.

All analyses were performed on an Ultimate 3000 Rapid Separation Liquid Chromatography (RSLC) system (Thermo Scientific) equipped with a ESI-Q-TOF mass spectrometer (microTOFQII, Bruker Daltonics). Separation was carried out on an Ascentis Express Fused-Core™ C18 column (100 × 2.1 mm i.d., 2.7 μm; Supelco) with its corresponding guard column (Ascentis express, 2.1 mm id × 50 mm, 2.7 μm, Supelco). A gradient elution program was developed to enable glycoalkaloid separation. The flow rate was set at 400 μl/min and the solvent system was (A) water containing 0.1% formic acid (*v*/v) and (B) acetonitrile (ACN) containing 0.1% formic acid (*v*/v). The elution program was: 2% B for 5 min, 50% B for 35 min, 100% B for 5 min, back to 2% B in 5 min, and conditioning for 2.5 min. The column oven was controlled at 35°C, and the autosampler at 6°C. Injection volume was set at 5 μl.

Before analyses, the mass spectrometer was calibrated in the external mode using a mix of known masses (ESI-L Low Concentration Tuning Mix, Agilent Technologies). High Resolution Mass Spectrometry (HRMS) data were acquired in positive ionization and in MS scan modes. The source temperature was set at 195°C, the capillary voltage at 3.8 kV, nebulizer gas (N_2) at 2.8 bars, and dry gas (N_2) at 9 L/min. Mass spectra acquisition was set at 5000 spectra/s on a mass range of 50–2000 *m/z*. LC-MS raw data were processed using Data Analysis 4.1 software (ESI Compass 1.5, Bruker Daltonique).

The two targeted glycoalkaloids, α-tomatine and tomatidine, were observed at *m/z* 1034.5550 and *m/z* 416.3543, respectively (see Supplementary material 1: Table S1). However, injection of α-tomatine produced two different peaks (α-tomatine 1 and 2, see supplementary Table 1) with different retention times but a similar pseudo-molecular ion and fragmentation pattern. Furthermore, dehydrotomatine also was observed in tomato leaf samples at *m/z* 1032.5377 and characterized by a typical fragment ion corresponding to [Tomatidenol + Gal + H] + at *m/z* 576.3876 as described by Cataldi et al. (2005). An ion extraction method using a mass range of 0.01 Da was used to quantify these four glycoalkaloids. To obtain the corresponding quantity in μg compounds per mg of leaf dry mass, the measured ion abundance was reported on a standard calibration curve for α-tomatine and tomatidine obtained in the same analysis and reprocessing conditions. Since a standard for dehydrotomatine was not commercially available, we could not calculate the quantities of this compound in the leaves, but reported ion abundance per mg of leaf dry mass representing relative amounts.

Plant Infestation Forty DAS (Supplementary material 1: Fig. S1), the 3rd fully-developed leaf from the apex was infested with one *T. absoluta* egg that had been oviposited less than 24 h earlier. Eggs were checked daily until larvae hatched. If an egg failed to hatch (< 5% of eggs in total), a newly-hatched larva (< 6 h old) was released on the leaf. To prevent larvae from escaping, each infested leaf was covered with a nylon mesh bag (0.2 mm × 30 × 24 cm). The *T. absoluta* infestation was performed on 12 plants per cultivar per treatment (96 plants). Forty-five DAS, the other group of 96 plants (12 plants per cultivar per treatment) was infested with *B. tabaci* first-instar crawlers (Supplementary material 1: Fig. S1). Six crawlers were released on the 3rd fully-developed leaf of each plant. They were checked under a microscope to ensure the insects had started walking and piercing leaf tissues; otherwise new insects were used to re-

Table 1 Effects of nitrogen, water, and cultivar on plant traits: (**A**) Factorial ANOVA to test effects of nitrogen, water, and cultivar on plant leaf-gas exchange parameters: Photosynthesis rate, transpiration rate, and stomatal conductance, and (**B**) factorial ANOVA to test the effects of nitrogen, water, and cultivar on leaf biochemistry: plant leaf N content, leaf C/N ratio, and glycoalkaloid concentrations (tomatidine, dehydrotomatine, α-tomatine 1, and α-tomatine 2). Significant effects are highlighted in bold

A: Leaf-gas exchange	Photosynthesis rate		Transpiration rate		Stomatal conductance	
Source of variation	$F_{1,74}$	*P*	$F_{1,74}$	*P*	$F_{1,74}$	*P*
Nitrogen	0.001	0.970	0.881	0.351	0.589	0.445
Water	**29.16**	**<0.001**	**4.625**	**0.035**	**8.389**	**0.005**
Cultivar	0.292	0.590	1.028	0.313	0.765	0.384
Nitrogen x Water	1.116	0.294	3.585	0.062	0.628	0.431
Nitrogen x Cultivar	1.830	0.180	0.002	0.965	0.304	0.583
Water x Cultivar	0.065	0.799	0.582	0.448	0.528	0.470

B: Leaf biochemistry	Leaf N content		Leaf C/N ratio		Tomatidine		Dehydrotomatine		α-tomatine 1		α-tomatine 2	
Source of variation	F_{1185}	*P*	F_{1185}	*P*	F_{1167}	*P*	F_{1167}	*P*	F_{1167}	*P*	F_{1167}	*P*
Nitrogen	**708.3**	**<0.001**	**501.4**	**<0.001**	**8.900**	**0.003**	**18.45**	**<0.001**	**5.020**	**0.026**	**6.540**	**0.012**
Water	3.650	0.058	0.300	0.585	**29.38**	**<0.001**	0.370	0.546	**12.60**	**0.001**	**7.160**	**0.008**
Cultivar	**7.140**	**0.008**	**6.140**	**0.014**	**50.71**	**<0.001**	**31.35**	**<0.001**	**8.870**	**0.003**	**12.30**	**0.001**
Nitrogen x Water	0.070	0.788	**4.090**	**0.045**	2.230	0.137	**3.890**	**0.005**	0.650	0.421	0.880	0.349
Nitrogen x Cultivar	**23.58**	**<0.001**	**22.08**	**<0.001**	3.590	0.060	**4.620**	**0.033**	0.060	0.809	**8.870**	**0.003**
Water x Cultivar	0.320	0.570	0.100	0.750	**11.12**	**0.001**	0.170	0.677	**7.500**	**0.007**	1.510	0.221

infest the same leaf from the same plant. All bio-assays were carried out under laboratory conditions (25 ± 2°C, RH 70 ± 10%, 16 h light) at INRA, Sophia-Antipolis, France.

Biological Traits of Insects For *T. absoluta*, survival and development time of larvae, pupae, and adults were recorded. Pupal mass was recorded by weighing pupa individually. The number of dead *B. tabaci* crawlers in the second or third instar stages was recorded one week after infestation.

Statistical Analysis We used *factorial ANOVAs* to test the effects of nitrogen (ON vs. LN), water (OW vs. LW) and cultivar type (cv. M vs. cv. NC) and all the possible interactions between these three main factors, on the plant traits separately, i.e., leaf photosynthesis rate, transpiration rate, stomatal conductance, leaf N content, leaf C/N ratio, and leaf glycoalkaloid concentrations, followed by *Tukey's post-hoc* tests for multiple comparisons among treatments. However, we found that none of the three-way interaction "nitrogen x water x cultivar" were significant; in addition, the results of these three-way interactions were not robust enough according to our analyses. Thus, only the results of two-by-two interactions are presented.

A *logistic regression* (log link function) was used to test the effects of nitrogen, water, cultivar type, and their interactions on the proportions of *T. absoluta* individuals developing from egg to pupa or to adult. A *factorial ANOVA* also was used to test the effects of nitrogen, water, and cultivar type on *T. absoluta* development time from egg to pupa or to adult as well as pupal weight, followed by multiple comparisons among the treatments for each trait using *Tukey's post-hoc* tests. A *logistic regression* also was used to test the effects of nitrogen, water, cultivar type, and their interactions on *B. tabaci* mortality, the numbers of individuals reaching the second or third instar. Multiple comparisons were made within each treatment to examine the differences among the three groups, i.e., the number of individual dead, reaching the second instar, or reaching the third instar. *Spearman rank correlation* was performed to test the relationship between leaf nitrogen content and the three whitefly groups using R software (R Core team, Vienna, Austria 2009). All other data were processed using SAS software (SAS Institute Inc 1999).

Results

Leaf-Gas Exchange Parameters Water input had a significant impact on plant photosynthesis rate, transpiration rate, and stomatal conductance (Table 1A). No significant interaction among the factors was found. However, under optimal nitrogen input, transpiration rate and stomatal conductance of cv. M was decreased under LW compared to OW input, whereas there were no effects on cv. NC (Fig. 1). For both cultivars, photosynthesis rate significantly decreased with decreasing water input (Table 1A; Fig. 1).

Leaf Nitrogen Content and C/N Ratio Nitrogen input had a strong impact on both leaf nitrogen content and C/N ratio, whereas water input did not (Table 1B). Plants receiving the LN treatment had significantly lower leaf nitrogen content and higher C/N ratio compared to plants receiving the ON treatment (Fig. 2). Furthermore, cv. NC responded more than cv. M under LN conditions compared to ON ones, regardless of water conditions (Significant effect of 'Cultivar' and 'Nitrogen x Cultivar' in Table 1; Fig. 2).

Glycoalkaloids Leaf glycoalkaloid concentration was higher in cv. M than in cv. NC. Limiting resource input tended to increase the concentration of these compounds in the leaf in both cultivars (Table 1B, Fig. 3). Under LN input, cv. NC had higher concentrations of tomatidine, dehydrotomatine, and α-tomatine 2 compared to ON treatment. However, in the cv. M, the concentration of all

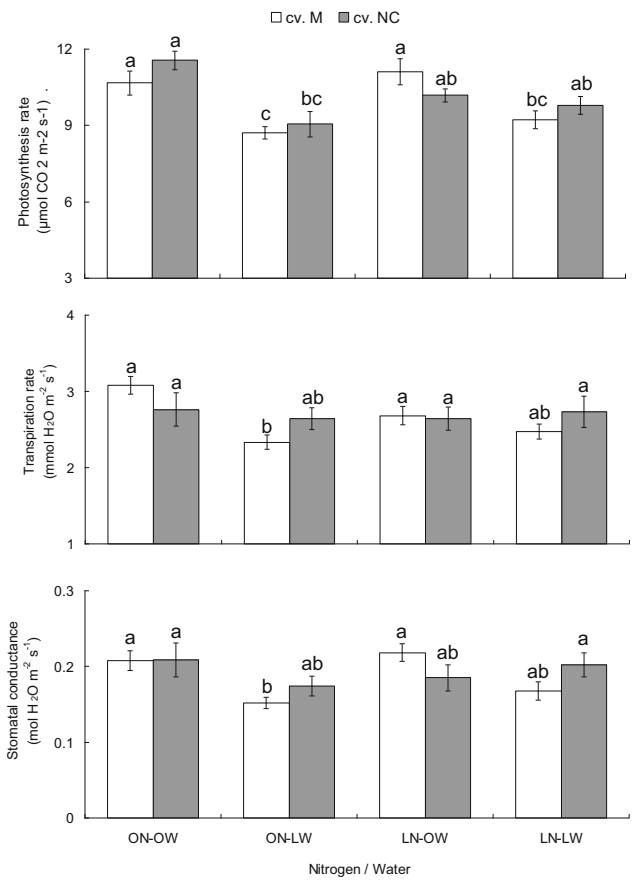

Fig. 1 Leaf photosynthesis rate, transpiration rate, and stomatal conductance (mean ± SEM, N = 9–11) measured for both tomato cultivars (M and NC) treated with optimal water input or limited water treatment (ON: optimal N; LN: limited N; OW: optimal water; LW: limited water). Different letters indicate significant difference ($P < 0.05$)

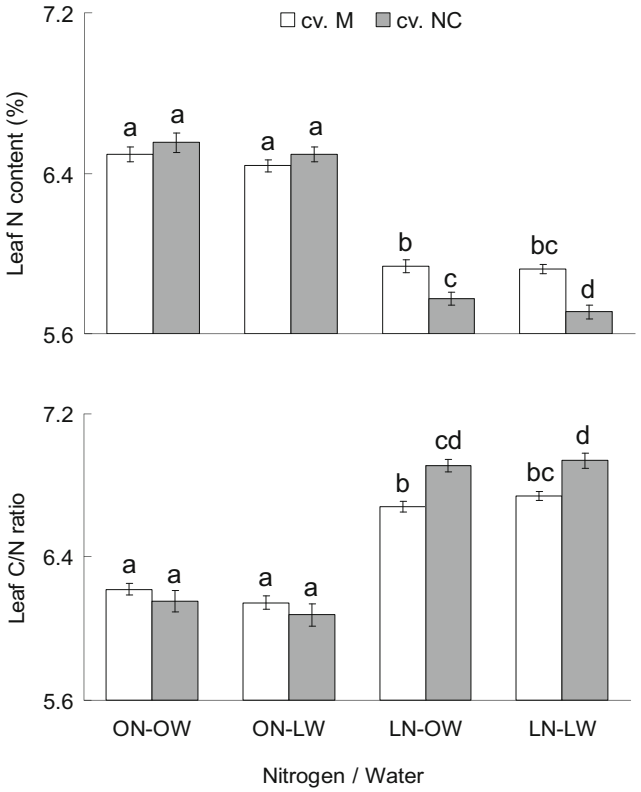

Fig. 2 Leaf nitrogen content (percent nitrogen in dry mass, mean ± SEM, $N = 24$) and leaf C/N ratio (unit-less, mean ± SEM, $N = 24$) for both tomato cultivars (M and NC) treated with different nitrogen and water inputs (ON: optimal N; LN: limited N; OW: optimal water; LW: limited water). Different letters indicate significant difference ($P < 0.05$)

glycoalkaloids did not respond to N treatment (Fig. 3). In contrast, cv. M showed a significant increase in concentrations of tomatidine and α-tomatine 1 under LW input, while leaf glycoalkaloid concentrations did not vary in response to water treatment in cv. NC (Fig. 3).

***Tuta absoluta* Survival and Development** Both nitrogen and water inputs significantly affected *T. absoluta* survival from egg to pupa or from egg to adult (Table 2A). The factor "cultivar" and the "cultivar × nitrogen" and "cultivar ×water" interactions had no significant effect on *T. absoluta* survival, thus indicating that the effect of nitrogen and water on *T. absoluta* survival was independent of cultivar type (Table 2A). In both cultivars, the survival proportion of *T. absoluta* from egg to pupa or from egg to adult decreased significantly with either LW or LN treatment compared to ON-OW treatment (Fig. 4).

Both nitrogen and water inputs significantly affected *T. absoluta* development time and pupal weight (Table 2A). Time from egg to pupa and time from egg to adult were both affected. The effects of "cultivar" and the interactions "cultivar × nitrogen" or "cultivar ×water" were not significant, indicating that the effects of nitrogen and water inputs on the three parameters were independent of cultivar type. The development time from egg to pupa or to adult was significantly prolonged when the plants grew under LN and/or LW treatment (other treatments vs. ON-OW, all $P < 0.05$) (Fig. 5). *Tuta absoluta* reached a significantly lower pupal weight on the plants grown under LN and/or LW treatment than on the ON-OW treatment (other treatments vs. ON-OW, all $P < 0.05$) (Fig. 5).

***Bemisia tabaci* Survival and Development** Nitrogen input affected *B. tabaci* mortality and the number of individuals reaching the third instar ($P < 0.001$ and $P = 0.006$, respectively), but not the number of individuals reaching the second instar ($P = 0.669$) (Table 2B). No effect of water was observed. The responses of *B. tabaci* mortality and development to nitrogen and water were similar between the cultivars. Limited nitrogen input increased mortality

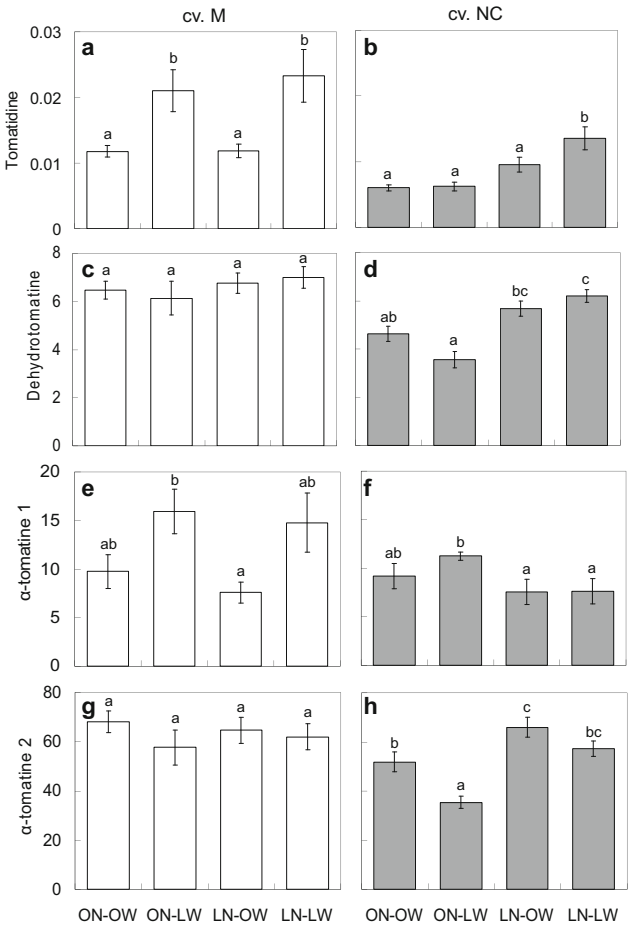

Fig. 3 Leaf glycoalkaloids concentration (mean ± SEM, $N = 24$): (**a** and **b**) tomatidine [μg/mg leaf dry mass (LDM)], (C and D) dehydrotomatine ($\times 10^4$) (relative content: ion abundance/mg LDM), (E and F) α-tomatine 1 (μg/mg LDM) and (G and H) α-tomatine 2 (μg/mg LDM) for both tomato cultivars (M and NC) treated with different nitrogen and water inputs. (ON: optimal N; LN: limited N; OW: optimal water; LW: limited water). Different letters indicate significant difference ($P < 0.05$)

Table 2 Effects of nitrogen, water, and cultivar on insect traits: (**A**) Logistic regression analysis testing the effects of nitrogen, water, cultivar, and their interactions on *Tuta absoluta* survival: Survival proportion from egg to pupa or to adult, and factorial ANOVA to test the effects of nitrogen, water, and cultivar on *T. absoluta* development: Development time from egg to pupa or to adult and pupal weight; (**B**) Logistic regression analysis to test the effects of nitrogen, water, cultivar, and their interactions on *Bemisia tabaci* survival: Number of dead individuals, or number of individuals reaching the second or third instar. Significant effects were highlighted in bold

A: *T. absoluta*	Survival proportion from egg to pupa ($df = 1$)		Survival proportion from egg to adult ($df = 1$)		Development time from egg to pupa		Development time from egg to adult		Pupal weight	
Source of variation	χ^2	P	χ^2	P	F_{1342}	P	F_{1263}	P	F_{1342}	P
Nitrogen	**17.02**	**<0.001**	**5.700**	**0.017**	**140.0**	**<0.001**	**85.32**	**<0.001**	**75.80**	**<0.001**
Water	**10.66**	**0.001**	**5.790**	**0.016**	**60.09**	**<0.001**	**44.49**	**<0.001**	**27.36**	**<0.001**
Cultivar	0.28	0.594	0.010	0.930	0.020	0.889	0.001	0.983	0.140	0.707
Nitrogen x Water	0.710	0.400	0.030	0.866	**10.23**	**0.002**	2.620	0.107	**13.80**	**<0.001**
Nitrogen x Cultivar	2.270	0.132	0.080	0.772	3.250	0.072	0.770	0.381	0.100	0.752
Water x Cultivar	0.160	0.686	0.230	0.632	1.470	0.226	1.110	0.294	0.180	0.674

B: *B. tabaci*		Number of dead individuals		Number of individuals reaching the second instar		Number of individuals reaching the third instar	
Source of variation	df	χ^2	P	χ^2	P	χ^2	P
Nitrogen	1	**17.37**	**<0.001**	0.18	0.669	**7.65**	**0.006**
Water	1	2.29	0.130	0.04	0.834	0.95	0.330
Cultivar	1	0.12	0.728	0.43	0.513	0.77	0.379
Nitrogen x Water	1	1.76	0.184	0.75	0.387	2.13	0.145
Nitrogen x Cultivar	1	0.03	0.871	0.00	0.998	0.00	0.956
Water x Cultivar	1	0.11	0.740	0.05	0.826	0.20	0.665

rate of *B. tabacci* (Fig. 6). Moreover, the number of dead individuals was negatively correlated with leaf N content (Fig. 7a), whereas the number of individuals reaching the third instar was positively correlated with leaf N content (Fig. 7b).

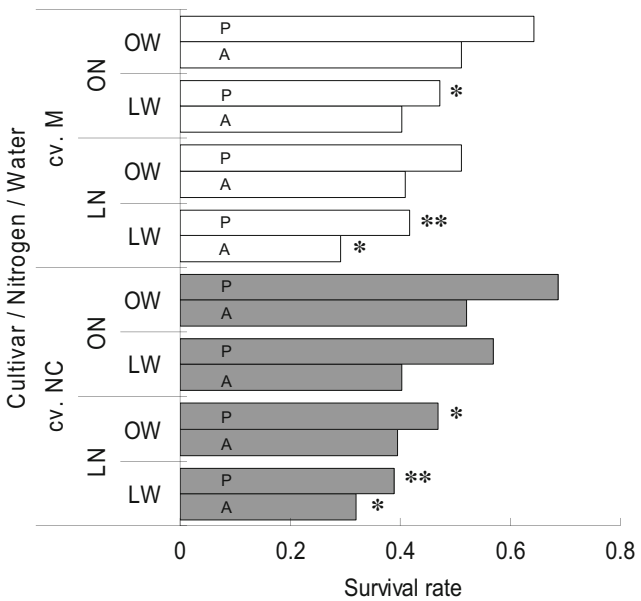

Fig. 4 Survival proportions ($N = 12$) of *Tuta absoluta* individuals from egg to pupa (P) or from egg to adult (A) on both tomato cultivars (M and NC) treated with different nitrogen and water inputs (ON: optimal N; LN: limited N; OW: optimal water; LW: limited water).ON-OW was considered as control group. * $P < 0.05$, ** $P < 0.01$ (significantly different from the survival rate of ON-OW group). Comparisons were done within cultivar either for pupae or adults using permuted Fisher exact test

Discussion

This study investigated whether plant cultivar differences modified the bottom-up effects of resource limitation on plant-insect herbivore interactions. The two cultivars exhibited different responses to resource restriction. Both herbivores from different feeding guilds responded differently to resource restriction: *T. absoluta* was affected negatively by nitrogen and water limitation, while *B. tabaci* was affected only by nitrogen limitation. However, cultivar differences were not strong enough to modify the bottom-up effects of nitrogen and water inputs on *T. absoluta* and *B. tabaci*. Our findings offer insights into the effects of nitrogen and water inputs on plant-insect herbivore interactions, a primary subject in the context of the rapidly accelerating environmental changes on local and global scales (Ballhorn et al. 2011; Bruce 2015; Lenhart et al. 2015).

Insects from Different Feeding Guilds Responded Differently to Nitrogen and Water Limitation Insect herbivores from different feeding guilds have different plant-feeding strategies and may respond differently to resource-stressed plants (Huberty and Denno 2004). In the current

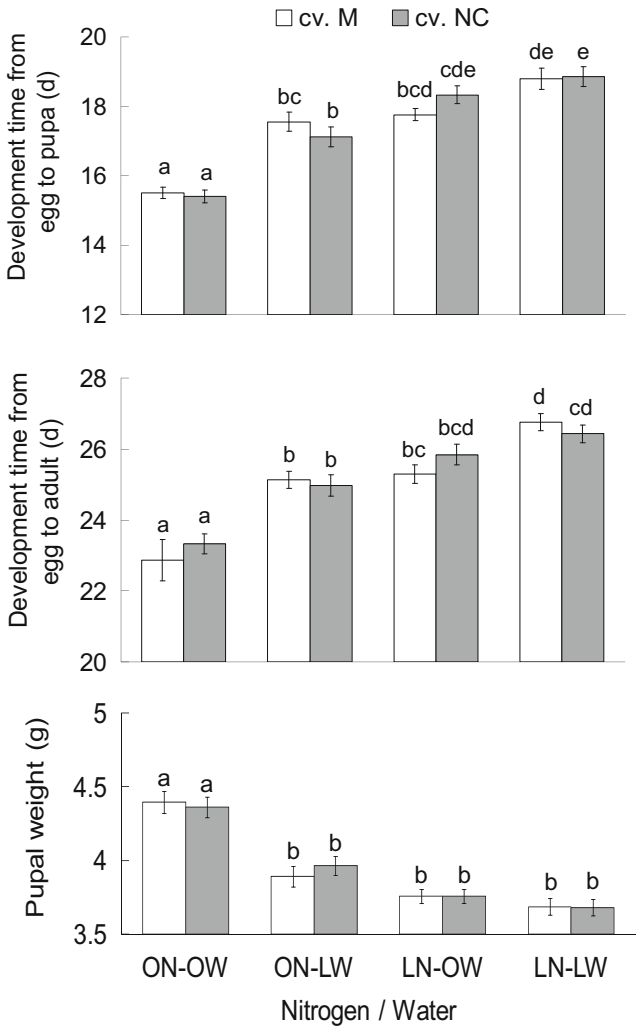

Fig. 5 *Tuta absoluta* development time (mean ± SEM, $N = 35–65$) from egg to pupa (d), development time (mean ± SEM, $N = 22–47$) from egg to adult (d), and pupal weight (mean ± SE, $N = 35–65$) (g) on both tomato cultivars (M and NC) treated with different nitrogen and water inputs (ON: optimal N; LN: limited N; OW: optimal water; LW: limited water). Different letters indicate significant difference at $P < 0.05$ by Tukey post hoc test for multiple comparisons

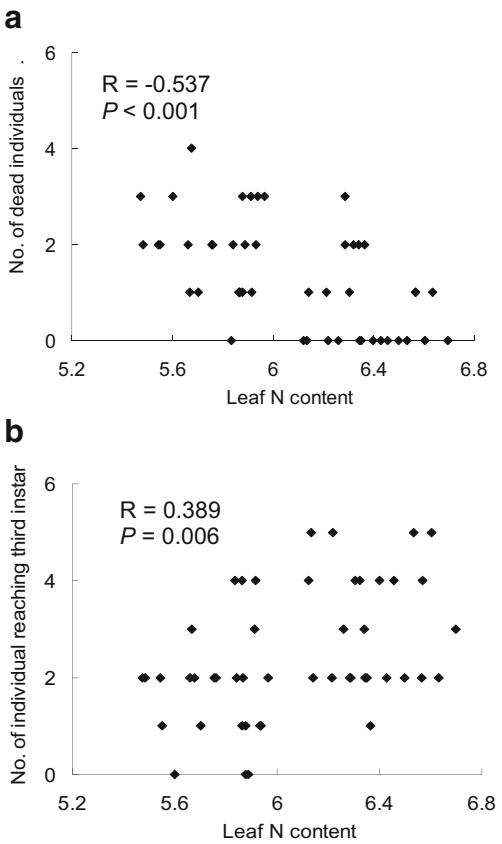

Fig. 7 Non-parametric correlations between leaf nitrogen content (% nitrogen in the dry mass) and (A) the number of dead *Bemisia tabaci* individuals or (B) those reaching the third instar on the corresponding plant. Data collected from cv. M and cv. NC were pooled. Spearman rank correlation was performed. R value refers to the correlation coefficient, and $P < 0.05$ denotes a significant correlation. The analysis on the number of *B. tabaci* individual reaching the second insar was not shown since the main effect of nitrogen on this parameter was not significant

study, both nitrogen and water shortages significantly affected *T. absoluta* survival and development, consistent with previous studies (Han et al. 2014). However, the phloem feeder *B. tabaci* was affected only by nitrogen limitation. The *nitrogen limitation hypothesis* (White 1993) predicts that insect

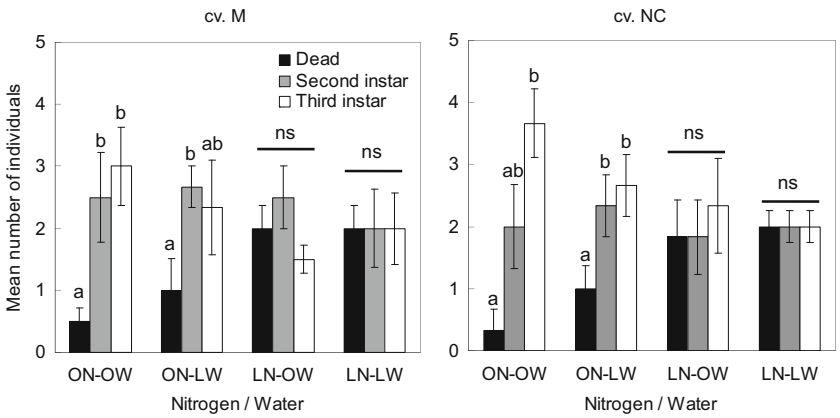

Fig. 6 Numbers of *Bemisia tabaci* individuals (mean ± SEM, $N = 12$) dead, reaching the second, or reaching the third instar on both tomato cultivars (M and NC) treated with different nitrogen and water inputs (ON: optimal N; LN: limited N; OW: optimal water; LW: limited water). Different letters indicate significant difference within each treatment combination ($P < 0.05$). ns: non-significant

herbivore performance will be positively correlated with leaf nitrogen content (Bi et al. 2003; Obermaier and Zwölfer 1999). The positive correlation between leaf nitrogen content and development of a chewing insect, specifically shorter development time but higher pupal weight for larvae feeding on plants with higher nitrogen content, has been documented (Han et al. 2014). Other studies have tested the correlation between plant N content and performance of phloem feeders. Inbar et al. (2001) did not find any correlation between leaf N content and oviposition behavior for *Bemisia argentifolii*, whereas Bi et al. (2003) found a positive correlation between petiole protein content and population densities of this species. In the present study, the positive correlation between nitrogen input and *B. tabaci* survival and development (Fig. 7) provided evidence to support the *nitrogen limitation hypothesis*. This example allows a broader generalization of this theory to include phloem-feeding insects.

Limited nitrogen input could have negative bottom-up effects on both insect herbivores by changing plant nutritional quality and plant defense. Insect herbivores need to consume large quantities of plant tissues to obtain sufficient nitrogen for optimal growth (Mattson 1980). Furthermore, a suitable plant protein to carbohydrate ratio in plant food is beneficial to growth and development for insect herbivores (Bede et al. 2007). Our data confirmed that leaf C/N significantly increased when less nitrogen was supplied (Inbar et al. 2001; Royer et al. 2013). The negative effects of nitrogen limitation on both insect herbivores may be due partly to decreased plant nutritional quality, i.e., an increase of C/N ratio (Fig. 2). The threshold at which leaf nutritional quality starts to have negative effects on insect herbivores ranged between $6 < C/N < 7$ for tomato plants under our conditions. In contrast, glycoalkaloids are a key chemical defense in Solanaceae, having negative impact on many insect herbivores (Altesor et al. 2014; Friedman 2002; Kowalski et al. 2000; Nenaah 2011). In our study, limited nitrogen input increased leaf glycoalkaloid concentrations in cv. NC, as has been shown in several studies (Larbat et al. 2012; Le Bot et al. 2009), and the concentrations were within the same variation range with Royer et al. (2013). Increased level of these compounds may be due to a larger allocation of resources to defense mechanisms involving N-based compounds than to growth under nitrogen limitation (Le Bot et al. 2009).

The negative bottom-up effects of water limitation on *T. absoluta* also were consistent with other studies on leaf-chewing insects (Gutbrodt et al. 2011; Inbar et al. 2001; Mody et al. 2009). Under water limitation, plant leaves may provide low-quality food to *T. absoluta* larvae for two reasons: (i) the larvae may struggle to obtain enough water for development; and (ii) the larvae may have limited access to nutrients due to decreased nitrogen utilization by plants (Slansky and Scriber 1985). Besides the lower nutritional value, water limitation often induces an increase in defensive compounds (Gutbrodt et al. 2011). Such a situation may have occurred on the cv. M, which showed an increased concentration of leaf glycoalkaloids (Fig. 3).

By contrast, *B. tabaci* was not affected by water limitation. Phloem feeders are unable to access the phloem sap if turgor is below a certain threshold (Huberty and Denno 2004). However, in our study, plants were only moderately water-stressed since they did not exhibit any noticeable symptoms of wilting. Thus, *B. tabaci* could obtain enough water from the phloem sap to fulfill their needs under water limitation. Finally, *B. tabaci* probably obtained sufficient nitrogen-based nutrients, such as free amino acids, since those compounds were still available in the phloem sap (Crafts-Brandner 2002).

Different Strategies to Cope with Nitrogen and Water Limitation in Cultivars Did not Modify the Negative Bottom-Up Effects Inter- and intra-specific plant variations in responses to environmental stresses are common (Barrett and Agrawal 2004). In our study, cv. M and NC responded differently to nitrogen and water limitation with regard to primary and secondary metabolic processes. On one hand, we found evidence that cv. M was more susceptible to water limitation. Well-N fertilized cv. M exhibited altered water status and increased glycoalkaloid concentrations, whereas cv. NC did not (Figs. 1 and 3). Moreover, our additional greenhouse trials highlighted the fact that cv. M was more sensitive to water limitation than cv. NC (Supplementary material 2). On the other hand, cv. NC appeared more susceptible to nitrogen limitation since leaf nitrogen based metabolisms varied with nitrogen input (Figs. 2 and 3). Such an effect on cv. NC was consistent with the findings of Royer et al. (2013), which documented that tomatine concentrations correlated positively with C/N in another tomato cultivar "Better Bush". Overall, these findings suggest that cv. NC is more susceptible to N limitation whereas cv. M appears more susceptible to water limitation, reflecting the fact that both genetic and environmental interactions determine the plant's chemical phenotypes (Ballhorn et al. 2011).

What is new in this study is the conclusion that the negative effects of resource limitation cascaded up on insect herbivores even though plant cultivars exhibited various adaptive traits to resource limitation. We conclude that the magnitude of plant trait variation (nutritional quality and defensive profile) in response to resource limitation was not sufficient to modify the bottom-up effects on herbivores. Perhaps the difference in plant nutritional quality between cultivars was too small to differentially affect the herbivores. Alternatively, the toxicity of glycolakaloids is structural and dose-dependent (Friedman 2002), and the concentrations of these compounds in our study may not have reached the toxicity threshold. Altesor et al. (2014) suggested that glycoalkaloids are less concentrated and varied in cultivated plant types than wild ones which are more resistant (as shown in the *Solanum* genus). Finally,

tomato plants have other defensive compounds that can impact insect herbivores such as phenolics, polyphenol oxidase, and protease inhibitors (Inbar et al. 2001; Larbat et al. 2015; Royer et al. 2013). Indeed, the latter study has shown that the negative bottom-up effects of nitrogen limitation on *T. absoluta* survival and development could be partly attributed to increased concentrations of phenolics in tomato plants.

The current study serves as a starting point for future efforts to unravel how inter-cultivar variations mediate plant-insect herbivore interactions. Further studies would need to include more cultivars and more herbivore species from various feeding guilds in order to apply our hypotheses more generally. From an applied perspective, plant-breeding programs that aim to address resource limitation can improve plant adaptability by selecting resistant cultivars to resource limitation. More interestingly, these cultivars can keep the negative bottom-up effect of resource limitation on insect herbivores. The concept may be helpful for optimizing future plant-breeding strategies by taking into account the function of pest management, especially for cultivars used in the arid areas or semi-barren land around the world, e.g., drought-prone environment (Galmés et al. 2013).

Acknowledgements This work was supported by the Chinese government (PhD fellowship to PH) and fund from FP7-PEOPLE-2012-IRSES, project ASCII [grant number: 318246]. We thank Philippe Bearez for technical assistance during the experiment, Vincent Calcagno and Louise Van Oudenhove de Saint Gery for suggestions on data analyses, the platform of analytical biochemistry (Sophia-Agrobiotech Institute Research; INRA), and the forest department of INRA Avignon (Ecologie des Forêts Méditerranéennes) for lending their Li-Cor equipment to us.

References

Altesor P, García Á, Font E, Rodríguez-Haralambides A, Vilaró F, Oesterheld M, Soler R, González A (2014) Glycolakaloids of wild and cultivated *Solanum*: effects on specialist and generalist insect herbivores. J Chem Ecol 40:599–608

Ballhorn DJ, Kautz S, Jensen M, Schmitt I, Heil M, Hegeman AD (2011) Genetic and environmental interactions determine plant defences against herbivores. J Ecol 99:313–326

Barrett RD, Agrawal AA (2004) Interactive effects of genotype, environment, and ontogeny on resistance of cucumber (*Cucumis sativus*) to the generalist herbivore, *Spodoptera exigua*. J Chem Ecol 30:37–51

Bede JC, McNeil JN, Tobe SS (2007) The role of neuropeptides in caterpillar nutritional ecology. Peptides 28:185–196

Bentz J, Reeves J III, Barbosa P, Francis B (1995) Nitrogen fertilizer effect on selection, acceptance, and suitability of *Euphorbia pulcherrima* (Euphorbiaceae) as a host plant to *Bemisia tabaci* (Homoptera: Aleyrodidae). Environ Entomol 24:40–45

Bi JL, Toscano NC, Madore MA (2003) Effect of urea fertilizer application on soluble protein and free amino acid content of cotton petioles in relation to silverleaf whitefly (*Bemisia argentifolii*) populations. J Chem Ecol 29:747–761

Bruce TJA (2015) Interplay between insects and plants: dynamics and complex interactions that have coevolved over millions of years but act in milliseconds. J Exp Bot 66:455–465

Cataldi TRI, Lelario F, Bufo SA (2005) Analysis of tomato glycoalkaloids by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Rapid Commun Mass Sp 19: 3103–3110

Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crop Res 105:1–14

Chailleux A, Desneux N, Seguret J, Maignet P, Khanh HDT, Tabone E (2012) Assessing European egg parasitoids as a mean of controlling the invasive south American tomato pinworm *Tuta absoluta*. PLoS ONE 7:e48068

Chailleux A, Biondi A, Han P, Tabone E, Desneux N (2013) Suitability of the pest-plant system *Tuta absoluta* (Lepidoptera: Gelechiidae) - tomato for *Trichogramma* (hymenoptera: Trichogrammatidae) parasitoids and insights for biological control. J Econ Entomol 106: 2310–2321

Chau A, Heinz KM, Davies FT Jr (2005) Influences of fertilization on population abundance, distribution, and control of *Frankliniella occidentalis* on chrysanthemum. Entomol Exp Appl 117:27–39

Chen Y, Olson DM, Ruberson JR (2010) Effects of nitrogen fertilization on tritrophic interactions. Arthropod-Plant Inte 4:81–94

Cornelissen T, Stiling P (2009) Spatial, bottom-up, and top-down effects on the abundance of a leaf miner. Ecography 32:459–467

Costamagna AC, Landis DA (2006) Predators exert top-down control of soybean aphid across a gradient of agricultural management systems. Ecol Appl 16:1619–1628

Crafts-Brandner SJ (2002) Plant nitrogen status rapidly alters amino acid metabolism and excretion in *Bemisia tabaci*. J Insect Physiol 48:33–41

Denno RF, Gratton C, Peterson MA, Langellotto GA, Finke DL, Huberty AF (2002) Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. Ecology 83:1443–1458

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29:185–212

Feng Y, Cao LY, Wu WM, Shen XH, Zhan XD, Zhai RR, Wang RC, Chen DB, Cheng SH (2010) Mapping QTLs for nitrogen-deficiency tolerance at seedling stage in rice (*Oryza sativa* L.). Plant Breed 129: 652–656

Fischer K, Fiedler K (2000) Response of the copper butterfly *Lycaena tityrus* to increased leaf nitrogen in natural food plants: evidence against the nitrogen limitation hypothesis. Oecologia 124:235–241

Friedman M (2002) Tomato glycoalkaloids: role in the plant and in the diet. J Agric Food Chem 50:5751–5780

Galmés J, Ochogavía JM, Gago J, Roldán EJ, Cifre J, Conesa MA (2013) Leaf responses to drought stress in Mediterranean accessions of *Solanum lycopersicum*: anatomical adaptations in relation to gas exchange parameters. Plant Cell Environ 36:920–935

Gutbrodt B, Mody K, Dorn S (2011) Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. Oikos 120:1732–1740

Han P, Lavoir AV, Le Bot J, Amiens-Desneux E, Desneux N (2014) Nitrogen and water availability to tomato plants triggers bottom-up effects on the leafminer *Tuta absoluta*. Sci Rep 4:4455

Han P, Bearez P, Adamowicz S, Lavoir AV, Desneux N (2015a) Nitrogen and water limitations in tomato plants trigger negative bottom-up effects on the omnivorous predator *Macrolophus pygmaeus*. J Pest Sci 88:685–691

Han P, Dong YC, Lavoir AV, Adamowicz S, Bearez P, Wajnberg E, Desneux N (2015b) Effect of plant nitrogen and water status on the foraging behavior and fitness of an omnivorous arthropod. Ecol Evol 5:5468–5477

Hogendorp BK, Cloyd RA, Swiader JM (2006) Effect of nitrogen fertility on reproduction and development of citrus mealybug, *Planococcus citri* Risso (Homoptera: Pseudococcidae), feeding on two colors of coleus, *Solenostemon scutellarioides* L. Codd. Environ Entomol 35: 201–211

Huberty AF, Denno RF (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. Ecology 85:1383–1398

Hunter MD, McNeil JN (1997) Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. Ecology 78:977–986

Inbar M, Doostdar H, Mayer R (2001) Suitability of stressed and vigorous plants to various insect herbivores. Oikos 94:228–235

Joern A, Behmer ST (1998) Impact of diet quality on demographic attributes in adult grasshoppers and the nitrogen limitation hypothesis. Ecol Entomol 23:174–184

Kowalski S, Domek J, Sanford L, Deahl K (2000) Effect of α-tomatine and tomatidine on the growth and development of the Colorado potato beetle (Coleoptera: Chyrosmelidae): studies using synthetic diets. J Entomol Sci 35:290–300

Larbat R, Le Bot J, Bourgaudm F, Robin C, Adamowicz S (2012) Organ-specific responses of tomato growth and phenolic metabolism to nitrate limitation. Plant Biol 14:760–769

Larbat R, Adamowicz S, Robin C, Han P, Desneux N, Le Bot J (2015) Interrelated responses of tomato plants and the leaf miner *Tuta absoluta* to nitrogen supply. Plant Biol 18:495–504

Le Bot J, Bénard C, Robin C, Bourgaud F, Adamowicz S (2009) The "trade-off" between synthesis of primary and secondary compounds in young tomato leaves is altered by nitrate nutrition: experimental evidence and model consistency. J Exp Bot 60:4301–4314

Lenhart PA, Eubanks MD, Behmer ST (2015) Water stress in grasslands: dynamic responses of plants and insect herbivores. Oikos 124:381–390

Mattson WJ (1980) Herbivory in relation to plant nitrogen content. Annu Rev Ecol 11:119–161

Mody K, Eichenberger D, Dorn S (2009) Stress magnitude matters: different intensities of pulsed water stress produce non-monotonic resistance responses of host plants to insect herbivores. Ecol Entomol 34:133–143

Mouttet R, Kaplan I, Bearez P, Amiens-Desneux E, Desneux N (2013) Spatiotemporal patterns of induced resistance and susceptibility linking diverse plant parasites. Oecologia 173:1379-1386

Nenaah G (2011) Individual and synergistic toxicity of solanaceous glycoalkaloids against two coleopteran stored-product insects. J Pest Sci 84:77–86

Obermaier E, Zwölfer H (1999) Plant quality or quantity? Host exploitation strategies in three Chrysomelidae species associated with Asteraceae host plants. Entomol Exp Appl 92:165–177

Price PW (1991) The plant vigor hypothesis and herbivore attack. Oikos 62:244–251

R Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Royer M, Larbat R, Le Bot J, Adamowicz S, Robin C (2013) Is the C:N ratio a reliable indicator of C allocation to primaryand defence-related metabolisms in tomato? Phytochemistry 88:25–33

Sarmento RA, Lemos F, Bleeker PM, Schuurink RC, Pallini A, Oliveira MGA, Lima ER, Kant M, Sabelis MW, Janssen A (2011) A herbivore that manipulates plant defence. Ecol Lett 14:229–236

SAS Institute Inc (1999) SAS/STAT User's Guide, Release 6.07, vol. 1. SAS Institute Inc.: Cary, NC

Slansky F Jr, Scriber JM (1985) Food consumption and utilization. In: Kerkut GA, Gilbert LI (eds) Comprehensive insect physiology, biochemistry, and pharmacology. Pergamon Press, Oxford, pp. 87–163

Tariq M, Wrignt DJ, Rossiter JT, Staly JT (2012) Aphids in a changing world: testing the plant stress, plant vigour and pulsed stress hypotheses. Agr Forest Entomol 14:177–185

White TCR (1993) The inadequate environment: nitrogen and the abundance of animals. Springer, Heidelberg

White TCR (2009) Plant vigour versus plant stress: a false dichotomy. Oikos 118:807–808