Increased water salinity applied to tomato plants accelerates the development of the leaf miner *Tuta absoluta* through bottom-up effects

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Variation in resource inputs to plants may trigger bottom-up effects on herbivorous insects. We examined the effects of water input: optimal water vs. limited water; water salinity: with vs. without addition of 100 mM NaCl; and their interactions on tomato plants (*Solanum lycopersicum*), and consequently, the bottom-up effects on the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Plant growth was significantly impeded by limited water input and NaCl addition. In terms of leaf chemical defense, the production of tomatidine significantly increased with limited water and NaCl addition, and a similar but non-significant trend was observed for the other glycoalkaloids. *Tuta absoluta* survival did not vary with the water and salinity treatments, but the treatment “optimal water-high salinity” increased the development rate without lowering pupal mass. Our results suggest that caution should be used in the IPM program against *T. absoluta* when irrigating tomato crops with saline water.

Plants are known to serve as a food source and shelter, thus ensuring survival and development of herbivorous insects\(^1\), although plants adopt various defensive strategies to cope with insect herbivory\(^2,3\). Plants, however, often grow in a variable environment where abiotic stress could be caused by cold, heat, drought, salt or chemical pollutants\(^4\). The environmental abiotic factors trigger changes in plant characteristics which can subsequently impact the performance of herbivorous insects. Such a cascading effect through plant-insect interactions is called the bottom-up effect\(^5–7\).

Water is crucial for plant growth, and water limitation can cause considerable changes in plant morphology, physiology and biochemistry\(^8\). Plants under drought conditions often impede survival and development of herbivorous insects because of the enhanced plant chemical defense\(^8\), and/or decreased nutritive quality of host as food\(^9,10\). However, there is no general consensus on the effects of water limitation on herbivorous insects since positive, negative and non-significant effects have all been documented\(^11\). Various factors have been shown to mediate the diversity of responses, such as the pattern of water limitation\(^11\), the feeding strategy adopted by insects\(^6\), e.g., chewing or sap-feeding insects, as well as feeding specialization\(^3\), e.g., specialist or generalist.

Plant-water relationships are often mediated by salinity status of irrigation, particularly in agro-ecosystems\(^12\). Salinity stress is considered a major environmental issue and a substantial constraint to plant growth\(^13\). One of the mechanisms may be that plants face lower water availability because of the increased salt concentration in the irrigation water\(^12,14\). Sodium chloride (NaCl) is one of the most common ingredients in soil or irrigation water causing salinity stress in plants\(^4\). NaCl stress can induce loss of intracellular water in plants. In addition, the effects of salinity on nitrogen metabolism are highly relevant since it may reflect osmotic and/or specific interactions of NaCl in several steps of nitrogen assimilation\(^13\). Increased soil salinity may induce changes in plant quality, especially in secondary metabolisim\(^15\); these changes may in turn have an impact on herbivorous insects through

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Table 1. Factorial ANOVAs to test the effects of “water”, “salt”, “insect (T. absoluta)”, and their interactions (if applicable) on (A) plant traits: plant height and number of nodes per plant on 34 DAS (DAS - days after sowing), concentrations of four glycoalkaloids: tomatidine, α-tomatine 1 and 2 and dehydrotomatine; (B) insect traits: T. absoluta pupal weight, development time from egg to pupa and development time from egg to adult. The statistical results of effects on T. absoluta survival are presented in the text.

| (A) Plant traits | Plant height | No. of nodes/plant | Tomatidine | α-tomatine 1 | α-tomatine 2 | Dehydrotomatine |
|------------------|--------------|--------------------|------------|--------------|--------------|----------------|
| Source of variation | F1,56 | P | F1,56 | P | F1,56 | P | F1,56 | P |
| water            | 81.33 <0.001 | 49.68 <0.001 | 4.940 0.029 | 1.476 0.228 | 2.168 0.145 | 2.326 0.134 |
| salt             | 64.41 <0.001 | 15.68 <0.001 | 8.264 0.005 | 2.565 0.113 | 3.327 0.072 | 2.480 0.119 |
| insect           | - - - -     | - - - -          | 1.278 0.262 | 0.314 0.576 | 0.450 0.265 | 0.300 0.258 |
| water x salt     | 0.310 0.577 | 0.010 0.916 | 2.997 0.087 | 0.576 0.450 | 1.260 0.265 | 1.300 0.258 |
| water x insect   | - - - -     | - - - -          | 0.707 0.403 | 0.108 0.744 | 0.199 0.657 | 0.249 0.619 |
| salt x insect    | - - - -     | - - - -          | 1.184 0.280 | 0.336 0.564 | 0.593 0.444 | 0.420 0.519 |
| water x salt x insect | - - - - | - - - -          | 0.489 0.486 | 0.154 0.696 | 0.333 0.566 | 0.285 0.595 |

| (B) Insect traits | Pupal weight | Development time from egg to pupa | Development time from egg to adult |
|------------------|--------------|----------------------------------|----------------------------------|
| Source of variation | F1,56 | P | F1,56 | P | F1,56 | P |
| water            | 7.169 0.010 | 2.258 0.139 | 3.179 0.082 |
| salt             | 0.253 0.617 | 8.789 0.004 | 6.607 0.014 |
| water x salt     | 1.206 0.277 | 1.737 0.193 | 1.717 0.197 |

bottom-up effects. However, little information is known about such bottom-up effects except those studies conducted in salt marsh systems where insects have been found to be positively\(^8\)\(^-\)\(^18\), negatively\(^9\), or neutrally\(^20\)\(^-\)\(^21\) influenced by increasing soil salinity. These differing results could be explained by the impact of variation in salinity stress tolerance in plants\(^15\), variation in predation pressure due to plant morphological characteristics\(^17\), as well as other abiotic features such as nitrogen fertilization\(^14\).

Unlike natural ecosystems where many salinity-tolerant species have evolved to adapt to high salinity\(^22\), many acclimated crop cultivars are not salinity-tolerant. This is especially true for those grown under controlled cropping systems, e.g., greenhouses. In these systems, economically dependent on certain leaf-mining insects, her-bivory by pest insects has long been a major agricultural challenge\(^10\). The larvae can penetrate and feed within the plant tissues and they are thus considered to have intimate relationship with their host plants\(^6\). However, the bottom-up effect of salinity stress on leaf-mining insects has rarely been documented. Moreover, the potential interactive effect of water and salinity stress on leaf-mining insects remains elusive.

With this context in mind, we examined the bottom-up effects of water and salinity on a leaf-mining insect in an agro-ecosystem. We evaluated the effects of water-salinity on plant growth and chemical defense traits, as well as on insect survival and development. Since strong bottom-up effects of soil nitrogen and water inputs on the performance of a leaf miner have been reported owing to the changes in plant nutritional quality as well as plant chemical defense\(^16\), we hypothesized that varying soil water/salinity may also trigger bottom-up effects on the leaf miner. To test this hypothesis, we set up a “water/salinity – plant – leaf miner” system using the tomato plant, Solanum lycopersicum L and the tomato leaf miner, Tuta absoluta Meyrick (Lepidoptera: Gelechiidae). Tomatoes are important greenhouse crops in many semi-arid regions where soil and groundwater salinity have been considered a major issue in crop production such as the Mediterranean region\(^12\)\(^-\)\(^23\). Tuta absoluta is a devastating pest threatening the worldwide tomato production\(^24\)\(^-\)\(^26\). This species has invaded Europe, rapidly spreading across the Mediterranean basin countries since its first appearance in Spain in 2006\(^27\). Since the invasion, the “tomato – leaf miner” system has been extensively studied\(^28\)\(^-\)\(^31\), notably in plant-insect interaction studies\(^30\)\(^,\)\(^32\).

**Results**

**Plant growth.** **Plant height.** Tomato plant height measured on 34 DAS (days after sowing) was significantly affected by water and salt treatments (Table 1A). No interaction of the two factors was found. In comparison to OW (optimal water) treatment, plant height decreased by 15.8%, 17.9% and 31.6% under the OW S+ (optimal water with high salinity), LW (limited water) and LW S+ (limited water with high salinity) treatments, respectively (Fig. 1).

**Number of nodes.** The number of nodes per plant showed a similar response pattern to water and salt treatments (Table 1A and Fig. 1). This trait differed significantly among the four treatments averaging 7.9, 7.4, 7.0 and 6.5 under OW, OW S+, LW and LW S+, respectively (Fig. 1).

**Plant defense.** Factorial ANOVAs suggested no significant effect of water, salt and their interactions on glycoalkaloids, except for one compound: tomatidine (Table 1A). The concentration of tomatidine increased significantly under LW S+ treatment compared to OW treatment, with intermediate levels for the other two treatments OW S+ and LW (Fig. 2). For the concentrations of the other three compounds measured, no marked difference was found among salinity-water treatments (despite similar trends than the one observed for tomatidine). The lowest level was obtained from the OW treatment, intermediate levels from the LW and OW S+, and the highest level from...
LW S+. Tuta absoluta herbivory did not induce changes in concentrations of the glycoalkaloids in leaves (“insect”: all $P > 0.05$ in Table 1A); therefore the data from the “With Tuta” and “Without Tuta” groups were pooled (Fig. 2).

**T. absoluta survival.** Neither water input nor salinity stress significantly affected $T.$ absoluta survival rate (water: $\chi^2 = 0.499$, df = 1, $P = 0.480$; salinity: $\chi^2 = 0.125$, df = 1, $P = 0.724$; interaction: $\chi^2 = 0.499$, df = 1, $P = 0.480$) (Fig. 3). $T.$ absoluta survival in response to water and salinity significantly depended on the developmental stages (stage: $\chi^2 = 6.210$, df = 1, $P = 0.013$). Overall, $T.$ absoluta survival rate from egg to pupa or to adult did not differ among the four treatment combinations within each group (Fig. 3).

**T. absoluta development.** *Pupal weight.* Water input significantly impacted $T.$ absoluta pupal weight (Table 1B), whereas salt addition and its interaction with water input did not impact pupal weight. $T.$ absoluta showed lower pupal weight on the plants treated with LW compared to the OW treatment (Fig. 4). The average pupal weight of the individual feeding on plants treated with OW, OW S+, LW and LW S+ was 3.91 g, 3.58 g, 3.15 g and 3.27 g, respectively.

Development time from egg to pupa or to adult. Salt addition had a significant effect on $T.$ absoluta development time from egg to pupa or to adult (Table 1B), whereas water input and its interaction with salinity stress did not. While the development time from egg to pupa or to adult averaged 18.4 and 25.3 days under the OW treatment, $T.$ absoluta exhibited shorter development times, i.e., 17.0 and 23.9 days under the OW S+ treatment, respectively (Fig. 4).

**Discussion**

Our study demonstrated that varying water-salinity treatments on tomato plants triggered bottom-up effects on plant-leaf miner interactions. $T.$ absoluta survival did not vary with the water and salinity treatments; however, the insect development rate increased without lowering pupal mass under the increased salinity conditions. Firstly, we demonstrated how plant growth and chemical defensive profiles were affected by various water and salinity treatments; then we explained how the changes in host plant nutritional and defensive features could explain the bottom-up effects on $T.$ absoluta.

The four water and salinity treatments resulted in a gradient of plant growth performance with the OW-treated plants being the highest with the most nodes and LW S+ treated ones being the smallest with the fewest nodes.
Figure 2. Effects of water and salt inputs on the concentrations of four glycoalkaloids in tomato leaves: tomatidine (μg/mg leaf dry mass (LDM)), α-tomatine 1 (μg/mg LDM), α-tomatine 2 (μg/mg LDM) and dehydrotomatine (x10^4) (relative content: ion abundance/mg LDM). OW: optimal water; LW: limited water; OW S+: optimal water and salinity stress (100 mM NaCl); LW S+: limited water and salinity stress. Histograms with different letters indicate significant difference at P < 0.05. Absence of letters indicates no significant difference among water and salinity treatments.
reduce more leaf glycoalkaloids under OW S plants. Nevertheless, this hypothesis may not be supported by our data as the tomato plants did not produce these compounds in lettuce40, b) phenolics, anthocyanins and flavones in sugarcane41, increased concentrations of other secondary metabolites in many plant species. The known examples are a) carotenoids, phenolics and antioxidative enzymes in Catharanthus roseus42, dimerpenes lactone, flavonoids and tannins in cotton 43 and lastly, 1,4-benzoxazin-3-one aglycones in maize 44. While water shortage has been shown to induce higher accumulation of leaf glycoalkaloids in tomato plants9, to our knowledge, this is the first time that salinity stress has been found to have an impact on the production of these compounds in tomato leaves. A similar effect of salinity has been observed on glycoalkaloids in the roots of another plant species, Catharanthus roseus45. Moreover, high salinity has recently been documented to induce increased concentrations of other secondary metabolites in many plant species. The known examples are a) carotenoids, phenolics and antioxidative enzymes in Catharanthus roseus45, b) phenolics, anthocyanins and flavones in sugarcane41, cyanogenic compounds in white clover45, c) various enzymatic defensive compounds in Andrographis paniculata46, d) diterpenes lactone, flavonoids and tannins in cotton47 and lastly, 1,4-benzoxazin-3-one aglycones in maize44. Increased production of these compounds could act to enhance chemical defense via their toxic activities15,40-44. In our study, however, the effect of water and salinity treatments was only seen on tomatidine even if a trend was observed for the other glycoalkaloids (Fig. 2). We assume the salt stress might be too weak to induce changes in glycoalkaloid concentrations in tomato leaves.

Although water shortage plus nutrient deficiency to plants (LW treatment) significantly reduced T. absoluta pupal weight, this treatment did not disrupt its development time. By contrast, T. absoluta development time was shortened when salt was added to the nutrient solution applied to plants (Fig. 4). We suggest that high soil salinity may influence T. absoluta development through three mechanisms. Firstly, salinity stress may affect T. absoluta development by reducing the availability of leaf water, since it can result in plant water deficit similar to a form of physiological drought45. In that case, T. absoluta larvae may face difficulties gaining sufficient water from tomato leaves. Secondly, salinity stress may affect the development of T. absoluta by enhancing the chemical defense of the host plants. Nevertheless, this hypothesis may not be supported by our data as the tomato plants did not produce more leaf glycoalkaloids under OW S+ treatment (Fig. 2) where a shorter development time in T. absoluta larvae was recorded (Fig. 4). Indeed, it has been suggested that glycoalkaloids are less concentrated and varied in cultivated plant species than in wild ones, as shown in the Solanum genus46. In our study, the only compound that varied with water and salinity, i.e., tomatidine, has been considered non-toxic for many herbivorous insects47. In addition, we explicitly acknowledge that tomatoes possess many other resistance-related traits that can impact their performance, including defensive compounds such as phenolics, e.g., chlorogenic acid, rutin and kaempferol and defense enzymes, e.g., polyphenol oxidase and protease inhibitor28,48. Thirdly, a possible explanation could be that excessive accumulation of Na+ and Cl– ions49 and cyanide15 in leaves may lower the suitability of leaf food for T. absoluta larvae. This hypothesis matches the results in Fig. 4 showing strong effects of salinity stress on T. absoluta development time under OW S+ treatment, whereas only a slight decrease was found under LW S+ treatment. Even with the same salt concentrations in both treatments, the absolute quantity of salts in OW S+ treatment was twice as high as in LW S+

An interesting question to consider is why T. absoluta managed to reach a normal pupal mass despite the fact that they underwent shorter larval development period under NaCl stress (Fig. 4). It has been acknowledged that insects may pupate with a lower mass accumulation if they have shortened plant resource consumption under adverse conditions. One hypothesis could be that T. absoluta larvae have to compensate for low leaf water content...
by accelerating their feeding rate\(^1\). It appears that *T. absoluta* larvae were able to employ different feeding strategies to adapt to varying lower plant food suitability, which means taking more time for larval development to compensate for nitrogen deficiency\(^9\), while spending less time for larval development to cope with salinity stress in the present study. Another assumption is that the larvae may benefit from higher concentrations of amino acids present in leaves to speed up their development. Indeed, higher proteolytic activities have been recorded in tomato leaves when tomato plants received 100 mM NaCl for 10 days\(^{13}\).

In conclusion, salinity stress has triggered strong bottom-up effects on *T. absoluta* development. These results highlight the importance of considering the salinity in irrigation for tomato crops when an IPM package has been designed to manage *T. absoluta*. In practice, a shorter development time, but unaffected survival rate and pupal mass accumulation in *T. absoluta*, may raise concern regarding higher damage by this pest when the tomato plants are grown under saline conditions. We predict that *T. absoluta* may have a greater population increase potential owing to their shorter life-cycle. This is more likely to occur in regions where the ground water, relatively high in salinity, is often used to irrigate tomato plants\(^{12,23}\). Therefore, to provide a full understanding of salt – tomato – *T. absoluta* interactions, future work is needed to monitor population dynamics of *T. absoluta* in multiple generations. Furthermore, the arthropod biological control agents from the higher trophic levels, the

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**Figure 4.** Pupal weight (mean ± SE, \(n = 13–15\)), development time from egg to pupa (mean ± SE, \(n = 13–16\)) and development time from egg to adult (mean ± SE, \(n = 11–13\)) of *T. absoluta* feeding on tomato plants treated with different water and salt inputs. OW: optimal water; LW: limited water; OW S+: optimal water and salinity stress (100 mM NaCl); LW S+: limited water and salinity stress. Histograms with different letters indicate significant difference at \(P < 0.05\).
parasitoids such as Necremnus sp., Trichogrammes and Braconidae species, and the predator such as Macrolophus pygmaeus, should be included in the testing system. This is due to the fact that salinity stress in plants may trigger bottom-up effects on the tritrophic interactions “plant-herbivorous insect-natural enemy”. This has been shown on other types of resources such as nitrogen.

Methods

Study organisms. The ‘Marmande’ tomato plant cultivar was grown in cubic plastic pots (7 x 7 x 6.5 cm) and kept in a climatic chamber (12 h light, 24 ± 1°C, 65 ± 5% RH). Tomato seedlings were then transferred into new pots containing limestone grains (Perlite Italiana srl, Corsico, Italy) mixed with nutrient soil on 6 DAS (days after sowing) (Fig. 5). On 24 DAS, we transferred the plants into larger pots (diameter: 10 cm, height: 9 cm) filled only with limestone grains.

The T. absoluta colony was reared on tomato plants in cages (40 x 40 x 40 cm) and kept in a climatic chamber (16 h light, 25 ± 1°C, 70 ± 10% RH). Honey and water were provided ad libitum as food source for T. absoluta adults. We collected 100 T. absoluta adults and put them into transparent plastic tubes (diameter: 3 cm, length: 10 cm) in order to gather the eggs for the subsequent experiments. Ten T. absoluta adults were released into each tube with one tomato leaflet put inside as the oviposition substrate. A total of ten tubes was prepared. Newly-oviposited T. absoluta eggs (≤ 24 h) were used to infest the plants.

Water and NaCl treatments. We set up the water and NaCl treatments by manipulating the quality and quantity of the stock nutrient solution which had been regularly used by our team to rear tomato plants in climatic chambers. The formula of this stock solution was prepared by mixing and diluting the following three concentrated solutions in a 100 L reserve stock, respectively: [Stock 1: HNO₃ (58 g/L) in 3 L, H₃PO₄ (75 g/L) in 1.5 L; Stock 2: KNO₃ = 7.5 kg, KH₂PO₄ = 3.5 kg, NO₃NH₄ = 0.5 kg, MgSO₄ = 1.5 kg, HNO₃ (58 g/L) in 50 mL, K₂SO₄ = 1 kg; Stock 3: KNO₃ = 3.75 kg, Ca(NO₃)₂ = 12.5 kg, Masquolate Fe in 2.8 L, HNO₃ (58% g/L) in 50 ml]. We carried out a full factorial design by combining the two levels of water treatment, i.e., optimal water vs. limited water input: “OW” vs. “LW”, and two levels of salinity treatment, i.e., with vs. without addition of 100 mM NaCl: “S+” vs. “blank,” to the plants starting on 24 DAS (Fig. 5). The final concentration of 100 mM in the nutrient solution was prepared to obtain S+ treatment as similar concentrations of NaCl had been used previously to create salinity stress on tomato plants. For the S+ treatment, the base nutrient solution with 100 mM NaCl was applied to the plants on a daily basis starting from 24 DAS until the T. absoluta pupated (Fig. 5). In order to acclimatize the plants to the NaCl stress, the nutrient solution in 50 mM NaCl was applied on two consecutive days before the nutrient solution in 100 mM NaCl was initiated. The nutrient solution, without NaCl, was thus used as the control (“Blank”). To differentiate water input, the volume of two types of nutrient solution was supplied in a “step increase” pattern throughout the tomato growing stage, following the protocols from our previous studies. Optimal daily water input, hereafter named “v” in volume of nutrient, was determined by the amount that fully saturates the perlite substrate without visible drainage, i.e., field capacity. The limited water treatment was determined by irrigating the plants with v/2 of the nutrient solution. We were unable to manipulate the water treatment by itself, thus it was accompanied by the simultaneous manipulation of nutrients inside the solution. In the latter case, the absolute quantity of nutrients in LW was half that in the OW treatment, but the concentration of nutrient solution remained constant among treatments. The LW treatment referred to half the volume of water as well as half the quantity of nutrients. A total of 88 plants was grown with 22 plants for each of the four treatments.

Plant traits. Plant growth and leaf sampling. We measured the plant height and counted the number of nodes per plant on 34 DAS.
To characterize the defense chemistry, the plants in two groups were sampled for the subsequent glycoalkaloids quantification (Fig. 3): (1) “Without Tuta”; leaf samples were collected on 48 DAS from *T. absoluta*-free plants with constitutive defense. Twelve plants were sampled, with three plants from each of the four treatments (*n* = 3); (2) “With Tuta”; leaf samples were collected from the *T. absoluta*-infested plants on 48 DAS when infested *T. absoluta* started to pupate, i.e., induced defense. Seventy-six plants in all were sampled with 19 plants from each of the four treatments (*n* = 19). We sampled all the leaves by cutting the fourth fully-developed leaves from the apex, the ones next to the third leaves that had been used for insect infestation (see below “Insect infestation”). The leaf samples were dried in a oven at 60 °C for 72 h and kept for further glycoalkaloid analyses.

**Glycoalkaloid analyses.** Glycoalkaloids act as key defensive compounds against various herbivorous insects in most plants from the Solanaceae family, e.g., tomato47,48,52,53. Glycoalkaloids were extracted from 5 mg dried tomato leaf powder mixed with 2 mL of 5% acetic acid (CH₃COOH) in water (v/v). The suspension was first mixed by vortexing and then extracted twice for 30 min using an ultrasonic assisted extractor at room temperature. After the extraction, the supernatant was filtered through a 0.45 μm PVDF PuradiscTM (Whatman, GE Healthcare). All samples were kept at −20 °C until analyzed. Glycoalkaloid standards (α-tomatine and tomatidine; Extrasynthese, Genay, France) were also diluted in a 5% CH₃COOH solution.

All analyses were performed on an Ultimate 3000 Rapid Separation LC (RSPLC) system (Thermo Scientific) equipped with a PDA detector and coupled to an 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 x 50 mM, 2.7 μm, Supelco) with its corresponding guard column (Ascentis express, 2.1 mM id x 50 mM, 2.7 μm, Supelco). An elution gradient was developed to separate glycoalkaloids. The flow rate was set at 400 μL/min and the solvent system was (a) water (H₂O) and formic acid (FA, 0.1% v/v) and (b) acetonitrile (ACN) 0.1% FA (v/v). The elution program was: 2% b for 5 min, 50% b for 35 min, 100% b for 5 min and thermostated for 3 min, back to 2% b in 5 min and conditioning for 2.5 min. The column oven was thermostated at 35 °C and the auto sampler at 6 °C. The injection volume was set at 5 μL.

Before analysis, the mass spectrometer was calibrated in an external mode using a mix of known masses (ESI-L Low concentration Tuning Mix, Agilent Technologies). 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 (N₂) at 2.8 bars and dry gas (N₂) at 9 L/min. Mass spectra acquisition was set at 5000 spectra/sec 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 respectively at m/z 1034.5550 and m/z 416.3543. However, injection of α-tomatine produced two different peaks (α-tomatine 1 and 2, see Supplementary Table S1) on our LC-MS platform which exhibited different retention times but a similar pseudo-molecular ion and fragmentation pattern. Furthermore, dehydrotomatine was also observed in tomato leaf samples at m/z 1032.5377 and characterized by a typical fragment ion corresponding to [Tomatidenol-H]+ at m/z 576.3876 as described by Cataldi et al.54.

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 of compounds per mg of leaf dry mass, the measured ion abundance was reported on a calibration curve for α-tomatine and tomatidine, obtained from 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. A relative quantification of this compound was then given in ion abundance per mg of leaf dry mass.

**Insect infestation.** On 30 DAS, the terminal leaflet of the third fully-developed leaf from the apex in each plant was chosen to be infested with one newly-oviposited *T. absoluta* egg, i.e., ≤24 h (Fig. 5). Nineteen plants were infested for each of the four treatments (*n* = 19). The eggs were monitored for the following four days since they took an average of four days to hatch under laboratory conditions (16 h light, 25 ± 1 °C, 60 ± 5% RH)19. If the eggs failed to hatch due to unintentional damage during the transfer, newly-hatched larvae were placed on the leaflets. To avoid larvae escaping, a plastic arena made from a 9-cm diameter petri-dish was used to trap each infested leaf. A 6-cm diameter hole on one side of the arena was covered by a 0.2 mM nylon mesh allowing ventilation. Such an arena design has been successfully used in our previous studies33,51.

**Insect traits.** The infested leaves were detached on 48 DAS, the same date as the leaf sampling for the glycoalkaloids measurement. The detached leaves with *T. absoluta* pre-pupae or pupae were maintained by inserting the stem into the sponge substrate saturated with water to maintain moisture for the insects. When all the individuals completely pupated, the leaves were removed and a small cotton ball saturated with water was placed in the arena to retain moisture. To estimate *T. absoluta* survival rate, the number of the individuals reaching pupal or adult stage was recorded. Pupal weight of each individual was measured when it had pupated completely. The development time from egg to pupa or to adult was recorded for each individual.

**Data analyses.** We firstly used MANOVAs to test the effects of water (limited water vs. optimal water input), salinity (with vs. without addition of salt), and/or insect (presence vs. absence of *T. absoluta* infestation) on the complex of variables, i.e., two and more dependent variables. The results showed significant effects of water and/or salinity on all the complex of variables, except for glycoalkaloids data. Hence separate factorial ANOVAs performed on each variable appeared to be justified. We subsequently performed factorial ANOVAs to test the effects of “water”, “salt”, “insect” and their interactions, if
applicable, on the four glycoalkaloids: tomatidine, α-tomatine 1, α-tomatine 2 and dehydrotomatine. Once a significant main effect of any factor was found in any trait, the differences among the four water and salinity treatments were tested using Tukey's post hoc test for multiple comparisons.

The Chi-square test was used to examine the effects of “water” and “salt” on \textit{T. absoluta} survival rates from egg to pupa or to adult. The factor “stage” was also tested to determine if \textit{T. absoluta} survival responded differently to the treatments according to different stages. The survival data was further analyzed with the permuted Fisher exact test. Factorial ANOVAs were performed to test the effects of “water”, “salt” and their interactions on a) \textit{T. absoluta} pupal weight, b) development time from egg to pupa and c) development time from egg to adult. Multiple comparisons within each trait were performed using Tukey’s post hoc test when the main effect was significant. All these data were computed using R software\textsuperscript{54}.

References
1. Schoonhoven, L. M., van Loom, J. A. & Dicke, M. \textit{Insect-plant biology}. (Oxford University Press, Oxford, 2005).
2. Howe, G. A. & Jander, G. Plant immunity to insect herbivores. \textit{Annu. Rev. Plant Biol.} 59, 41–66 (2008).
3. Dicke, M. & Baldwin, I. T. The evolutionary context for herbivore-induced plant volatiles: beyond the cry for help. \textit{Trends Plant Sci.} 15, 167–175 (2010).
4. Mahajan, S. & Tuteja, N. Cold, salinity and drought stresses: an overview. \textit{Arch. Biochem. Biophys.} 444, 139–158 (2005).
5. Hunter, M. D. & Price, P. W. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. \textit{Ecology} 73, 724–732 (1992).
6. Inbar, M., Doostdar, H. & Mayer, R. Suitability of stressed and vigorous plants to various insect herbivores. \textit{Oikos} 94, 228–235 (2001).
7. Denno, R. F. \textit{et al.} Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. \textit{Ecology} 83, 1443–1458 (2002).
8. Chaves, M. \textit{et al.} How plants cope with water stress in the field. Photosynthesis and growth. \textit{Ann. Bot.} 89, 907–916 (2002).
9. Gutbrod, R., Mody, K. & Dorn, S. Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. \textit{Oikos} 120, 1732–1740 (2011).
10. Han, P., Lavois, A. V., Le Bot, J., Amiens-Desneux, E. & Desneux, N. Nitrogen and water availability to tomato plants triggers bottom-up effects on the leaffminer \textit{Tuta absoluta}. \textit{Sci. Rep.} 4, 4455 (2014).
11. Huberty, A. F. & Denno, R. F. Plant water stress and its consequences for herbivorous insects: a new synthesis. \textit{Ecology} 85, 1383–1398 (2004).
12. Romero-Aranda, R., Soria, T. & Cuartero, J. Tomato plant-water and plant-water relationships under salinity growth conditions. \textit{Plant sci} 160, 265–272 (2001).
13. Debouba, M., Gouia, H., Suck, R. & Ghorbel, M. H. NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato “Lycopersicon esculentum”. \textit{J. Plant. Physiol.} 163, 1247–1258 (2006).
14. Soria, T. & Cuartero, J. Tomato fruit yield and water consumption with salty water irrigation. \textit{Acta Hortic.} 458, 215–219 (1997).
15. Ballhorn, D. J. & Elias, J. D. Salinity-mediated cyanogenesis in white clover (Trifolium repens) affects trophic interactions. \textit{Ann. Bot.} 114, 357–366 (2014).
16. Ellison, A. M. & Farnsworth, E. J. Seeding survivorship, growth, and response to disturbance in Belizean Mangal. \textit{J. Bot.} 80, 1137–1145 (1999).
17. Hacker, S. D. & Bertness, M. D. A herbivore paradox: why salt marsh marshes live on poor quality plants. \textit{Am. Nat.} 145, 192–210 (1995).
18. Rand, T. A. Variation in insect herbivory across a salt marsh gradient influences plant survival and distribution. \textit{Ecology} 132, 549–558 (2002).
19. Hemminga, M. A. & van Soelen, J. Estuarine gradients and the growth and development of \textit{Agapanthia villosowirensides} (Coleoptera), a stem borer of the salt marsh halophyte \textit{Aster tripolium}. \textit{Oecologia} 77, 307–312 (1988).
20. Hemminga, M. A. & van Soelen, J. The performance of the leaf mining microlepidopteran \textit{Bucculatrix maritime} (Sttt.) on the salt marsh halophyte, \textit{Aster tripolium} (L.), exposed to different salinity conditions. \textit{Oecologia} 89, 422–427 (1992).
21. Bowdish, T. I. & Siling, P. The influence of salt and nitrogen on herbivore abundance: direct and indirect effects. \textit{Oecologia} 113, 400–405 (1998).
22. Parida, A. K. & Das, A. B. Salt tolerance and salinity effects on plants: a review. \textit{Exotoc. Environ. Safe.} 60, 324–349 (2005).
23. Cuartero, J. & Fernández-Muñoz, R. Tomato and salinity. \textit{Sci. Hortic.} 78, 125 (1999).
24. Desneux, N. \textit{et al.} Biological invasion of European tomato crops by \textit{Tuta absoluta}: ecology, geographic expansion and prospects for biological control. \textit{J. Pest Sci.} 83, 197–215 (2010).
25. Desneux, N., Luna, M. G., Guillemaud, T. & Urbaneja, A. The invasive South American tomato pinworm, \textit{Tuta absoluta}, continues to spread in Afro-Eurasia and beyond – the new threat to tomato world production. \textit{J. Pest Sci.} 84, 403–408 (2011).
26. Zappalà, L. \textit{et al.} Natural enemies of the South American moth, \textit{Tuta absoluta}, in Europe, North Africa and Middle East, and their potential use in pest control strategies. \textit{J. Pest Sci.} 86, 635–647 (2013).
27. Biondi, A. \textit{et al.} Indigenous natural enemies attacking \textit{Tuta absoluta} (Lepidoptera: Gelechiidae) in Southern France. \textit{Egyt J. Biol. Pest Co.} 23, 117–121 (2013).
28. Jaworski, C. C., Chailleux, A., Bearez, P. & Desneux, N. Apparent competition between major pests reduces pest population densities on tomato crop, but not yield loss. \textit{J. Pest Sci.} 88, 793–803 (2015).
29. Van Damme, V. \textit{et al.} Overwintering potential of the invasive leaffminer \textit{Tuta absoluta} (Meyrick) (Lepidoptera: Gelechiidae) as a pest in greenhouse tomato production in Western Europe. \textit{J. Pest Sci.} 88, 533–541 (2015).
30. Lee, M. S., Albajes, R. & Eizaguirre, M. Mating behaviour of female \textit{Tuta absoluta} (Lepidoptera: Gelechiidae): polyanry increases reproductive output. \textit{J. Pest Sci.} 87, 429–439 (2015).
31. Campos, M. R., Silva, T. B., Silva, W. M., Silva, J. E. & Siqueira, H. A. A. Spinosyn resistance in the tomato borer \textit{Tuta absoluta} (Meyrick) (Lepidoptera: Gelechiidae). \textit{J. Pest Sci.} 85, 405–412 (2015).
32. Larbat, R. \textit{et al.} Interrelated responses of tomato plants and the leaffminer \textit{Tuta absoluta} to nitrogen supply. \textit{Plant biology}, doi: 1111/j. pl12425 (2015).
33. Han, P., Bearez, P., Adamowicz, S., Lavois, A. V. & Desneux, N. Nitrogen and water limitations in tomato plants trigger negative bottom-up effects on the omnivorous predator \textit{Macrophilus pygmaeus}. \textit{J. Pest Sci.} 88, 685–691 (2015).
34. Wang, W., Vinocur, B. & Altman, A. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. \textit{Planta} 218, 1–14 (2003).
35. Gouia, H., Ghorbel, M. H. & Touraine, B. Effects of NaCl on flows of N and mineral ions and NO\textsubscript{3} – reduction rate within whole plants of salt-sensitive bean and salt-tolerant cotton. \textit{Plant Physiol.} 105, 1409–1418 (1994).
36. Flores, P., Botella, M. A., Martínez, V. & Cerdá, A. Ionic and osmotic effects of nitrate reductase activity in tomato seedlings. \textit{J. Plant Physiol.} 156, 552–557 (2000).
37. Flores, P., Navarro, J. M., Carvajal, M., Cerdá, A. & Martínez, V. Tomato yield and quality as affected by nitrogen source and salinity. *Agronomie* **23**, 249–256 (2003).
38. Flores, P., Botella, M. A., Cerdá, A. & Martínez, V. Influence of nitrate level on nitrate assimilation in tomato (*Lycopersicon esculentum*) plants under saline stress. *Can. J. Bot.* **82**, 207–213 (2004).
39. Jaleel, C. A., Sankar, B., Sridharan, R. & Panneerselvam, R. Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in *Catharanthus roseus*. *Turkish J. Biol.* **32**, 79–83 (2008).
40. Mahmoudi, H. et al. The impact of genotype and salinity on physiological function, secondary metabolite accumulation, and antioxidative responses in lettuce. *J. Agr. Food Chem.* **58**, 5122–5130 (2010).
41. Wahid, A. & Ghazanfar, A. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. *J. Plant Physiol.* **163**, 723–730 (2006).
42. Shao, Y. H. et al. Effect of salt treatment on growth, isoenzymes and metabolites of *Andrographis paniculata* (Burm. f.) Nees. *Acta Physiol. Plant.* **37**, 35 (2015).
43. Wang, Q., Eneji, A. E., Kong, X., Wang, K. & Dong, H. Salt Stress Effects on Secondary Metabolites of Cotton in Relation to Gene Expression Responsible for Aphid Development. *Plos One* **10**, e0129541 (2015).
44. Forieri, I., Hildebrandt, U. & Rostás, M. Salinity stress effects on direct and indirect defence metabolites in maize. *Environ. Exp. Bot.* **122**, 68–77 (2016).
45. Tucker, S. S., Craine, J. M. & Nippert, J. B. Physiological drought tolerance and the structuring of tallgrass prairie assemblages. *Ecosphere* **2**, art48 (2011).
46. Altesor, P. et al. Glycolalkaloids of wild and cultivated Solanum: effects on specialist and generalist insect herbivores. *J. Chem. Ecol.* **40**, 599–608 (2014).
47. Friedmann, M. Tomato glycoalkaloids: role in the plant and in the diet. *J. Agr. Food Chem.* **50**, 5751–5780 (2002).
48. Royer, M., Larbat, R., Le Bot, J., Adamowicz, S. & Robin, C. Is the C:N ratio a reliable indicator of C allocation to primary and defence-related metabolisms in tomato? *Phytochemistry* **88**, 25–33 (2013).
49. Manaa, A. et al. Salt and genotype impact on plant physiology and root proteome variations in tomato. *J. Exp. Bot.* **62**, 2797–2813 (2011).
50. Chen, Y., Olson, D. M. & Ruberson, J. R. Effects of nitrogen fertilization on tritrophic interactions. *Arthropod-Plant Inte.* **4**, 81–94 (2010).
51. Han, P. et al. Effect of plant nitrogen and water status on the foraging behavior and fitness of an omnivorous arthropod. *Ecol. Evol.* **5**, 5468–5477 (2015).
52. Eich, E. *Solanaceae and Convolvulaceae: Secondary metabolites*. (Springer, Berlin-Heidelberg, 2008).
53. Cataldi, T. R. I., Lelario, F. & Bufo, S. A. Analysis of tomato glycoalkaloids by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Sp.* **19**, 3103–3110 (2005).
54. R Development Core Team. A language and environment for statistical computing. *R foundation for statistical computing. http://www.r-project.org/* (2009).

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**Author Contributions**
P.H., Z.-j.W. and N.D. scoped and designed the study; P.H., Z.-j.W., W.-y.Z., T.M., A.S. and A.-V.L. performed the experiments and analyzed data; P.H., A.-V.L., C.-y.N. and N.D. interpreted results and wrote the manuscript.

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Corrigendum: Increased water salinity applied to tomato plants accelerates the development of the leaf miner *Tuta absoluta* through bottom-up effects

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This Article contains typographical errors. In the Results section under subheading ‘*T. absoluta* development’,

“The average pupal weight of the individual feeding on plants treated with OW, OW S+, LW and LW S+ was 3.91 g, 3.58 g, 3.15 g and 3.27 g, respectively”.

should read:

“The average pupal weight of the individual feeding on plants treated with OW, OW S+, LW and LW S+ was 3.91 mg, 3.58 mg, 3.15 mg and 3.27 mg, respectively”.

In Figure 4, the y-axis ‘Pupal weight (mg)’ is incorrectly given as ‘Pupal weight (g)’. The correct Figure 4 appears below as Figure 1.
Figure 1.