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Changes in tolerance and resistance of a plant to insect herbivores under variable water availability

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ABSTRACT

Water availability is an important factor that influences plant-insect interactions. While the influence of water limitation on plant resistance traits has received much attention, how water availability affects plant tolerance to herbivory is rarely tested. Here we show that lower water availability reduced tolerance capacity of tomato plants as measured by above ground regrowth and flower development after herbivory. In contrast to a reduced ability to tolerate herbivory, lower water availability increased the constitutive and induced levels of two defensive proteins, trypsin protease inhibitor and polyphenol oxidase, indicative of an increased investment on resistance under water limitation. The increase in defense proteins was paralleled with lower performance of a specialist caterpillar, *Manduca sexta,* and lower consumption of plant tissues. Although the performance of generalist, *Helicoverpa zea*, was unaffected by water availability, we observed a high mortality of *H. zea* that suggests strong resistance of tomato against *H. zea*. The findings revealed an unexpected case where water limitation decreases tolerance but increases resistance of a plant, suggesting a potential tradeoff between these strategies. This plasticity may benefit herbaceous plants by balancing growth and defense under variable water availability.

1. Introduction

Changes in water availability have been identified to be a major factor that influences plant traits and affects the interactions between plants and insect herbivores (Mattson and Haack, 1987). Studies that investigate the underlying mechanisms have led to the proposal of several hypotheses, including the Plant Stress Hypothesis (White, 1974), the Plant Vigor Hypothesis (Price, 1991), and the Pulse Stress Hypothesis (Huberty and Denno, 2004). Most of these studies have focused mainly on changes in resistance traits of plant, such as secondary metabolites (Orians et al., 2019; Pineda et al., 2016), phytohormones (Xie et al., 2020); and it is commonly reported that the experimental water stress leads to an increased resistance, especially in herbaceous plants (Waring and Cobb, 1992). Although much is known about how water availability changes the resistance of plant against herbivore, there is a surprisingly lack of knowledge on how water availability affects other defense strategies against herbivory, such as tolerance (Jamieson et al., 2012).

Tolerance is an important defense strategy of plants that is influenced by water availability due to its close association with growth (Gianoli and Salgado-Luarte, 2017; Strauss and Agrawal, 1999). Although the impacts of water availability associated changes in tolerance seems less direct on insect herbivores compared to resistance, changes in tolerance can affect many important aspects of plant-insect interactions, such as changes in food availability for herbivores (Mcnaughton, 1983), plant competitiveness (Wise and Abrahamson, 2007), and population dynamics of both plants (Maron and Crone, 2006) and insects (Dixon and Kindlmann, 1990). As a result, changes in water availability can potentially lead to a community-wide impact by altering plant tolerance, and its importance will likely increase under the predictions of climate change model (National Research Council, 2011). However, there is a surprisingly lack of studies (but see Atala and Gianoli, 2009 and Sun et al., 2010) on interactions between tolerance and water availability.

Studies that investigated availability of other resources, such as light and nutrients, have contributed to much of our understanding of how resource availability affect tolerance of plants to herbivory (Wise and Abrahamson, 2007). The Limiting Resource Model predicts that the level

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of tolerance is affected by the importance of the limiting resource and how herbivory affects the acquisition of such resource (Wise and Abrahamson, 2007). Specifically, low water availability has been predicted to increase tolerance to leaf herbivory because (1) leaf herbivory does not affect the acquisition of focal resource (i.e., water), and (2) low focal resource limits plant fitness. It was also found in some cases that water stress leads to higher tolerances to artificial defoliation (Gassmann, 2004).

We hypothesize that low water availability might increase tolerance as well as resistance of plants to herbivory. To test this hypothesis, we use tomato and a destructive defoliator, tobacco hornworm (*Manduca sexta*), to investigate the influence of water availability on tolerance of tomato to herbivore damage; and by adding another important pest, tomato fruitworm (*Helicoverpa zea*), we investigate the influence of water availability on resistance of tomato to insect herbivores. Vegetative (i.e., regrowth) and reproductive parameters (i.e., flower, fruit, seed production) were selected to estimate tolerance, whereas herbivore performance and constitutive/induced defenses were analyzed to estimate resistance. Plants are known to possess a myriad of resistance traits, which can be effective depending on the species of herbivore (Gatehouse, 2002). Unlike tolerance traits which are relatively general, resistance traits can be specific, thus it is important to analyze the defense responses that are known to be adaptive against specific herbivores (Acevedo et al., 2015; Howe and Jander, 2008). Among the limited systems that have been studied in such details, tomato (*Solanum lycopersicum*) was selected because: (1) Tomato is well-defended, only a few insect herbivores frequently feed on tomato, (2) comparatively, large amount of information on inducible resistance traits (such as the defensive protein, trypsin protease inhibitor and polyphenol oxidase) against these insect herbivores and regulatory systems underlying these resistance traits have been reported (Chung and Felton, 2011; Pena-Cortes et al., 1995; Tian et al., 2014, 2012; Wolfson and Murdock, 1990).

2. Materials and methods

2.1. Plants and insects

Tomato, *S. lycopersicum* (cv. Better Boy), was grown in a greenhouse (Pennsylvania State University, USA) with artificial illumination and partial natural light under 16 h: 8 h (light/dark) photoperiod and at an ambient temperature of 27 °C/25 °C (light/dark). Seedlings were planted in 3.5 inch-pots (530 cm^3) with Metromix 400 potting mix, and fertilized twice at one- and four-leaf stage with 20−20−20 Scotts Peter Professional fertilizer (Griffin Greenhouse & Nursery Supplies, USA). Eggs of *Helicoverpa zea* and *Manduca sexta* were purchased from Benzon Research (Carlisle, USA) and Great Lakes Hornworm (Washington, USA), respectively. Larvae were reared on wheat germ/casein-based diet (Peiffer and Felton, 2009) purchased from BIOSERV (Frenchtown, USA) and maintained under the same photoperiod and temperature conditions as for plants.

2.2. Water status quantification

To create plants of variable water status, four-leaf stage tomato seedlings were subjected to a series of water treatments ranging from 10 mL to 200 mL per day. Three levels (a high and two lower) of water availability, (1) 200 mL/day, (2) 50 mL/day, and (3) 10 mL/day, were selected according to the changes in phenotype and biomass indicating distinct water statuses. Water was provided at 9 a.m., from a 1 mL pipette tip (cut off 2 cm from the narrow end) inserted into the soil right next to the plant for seven days. To quantify the water status, we took several indirect measurements of water stress (Jones, 2007), including height (cotyledons to the apical meristem), leaf number, and fresh weight of shoot, dry weight of shoot and root (70°C, 72 h), and soil volumetric water content (VWC, θ_v). Root to shoot ratio was calculated as: dry weight of root/ dry weight of shoot.

Leaf water status was monitored by recoding changes in leaf thickness using a device called “Leafy” developed by Afzal et al. (2017). This method has been shown to accurattely reflect the level of drought stress in tomato. To investigate the impacts of water treatments on physiological traits of plants, photosynthetic activity, stomatal conductance, and transpiration rate were determined using the LI-6400 portable photosynthesis system (Li-Cor, Lincoln, USA). The chamber light intensity of LI-6400 portable photosynthesis system was set to 1500 $\mu mol/m^2/sec$. The reference CO_2 concentration was set to 400 μmol/mol and vapor pressure deficit was set to 1. The flow rate was set at 500 μmol/sec. Measurements were done on both local and systemic leaves. The youngest mature leaves (the fourth fully expanded compound leaf counted from the bottom) were used as the “local leaf” (also for leaf thickness measurements, herbivore damage treatments, and leaf tissue collections). The leaf located two leaves above the local leaf is considered the “systemic leaf” (sixth fully expanded compound leaf counted from the bottom). Terminal leaflets were used in both local and systemic leaves. Number of replicates for all experiments can be found in the relevant figure legends.

2.3. Tolerance: compensatory regrowth and reproductive traits

To assess tolerance capacity of plants, we measured above ground regrowth and changes in reproductive outputs after herbivory under different levels of water availability. For the first set of experiments, we started with investigating the impacts of standardized artificial damage on regrowth capacity. All tissues above the second mature leaf (counting from bottom) were artificially removed with dissecting scissors. Plants were then kept under their original water regime as described above for 10 days. We then collected all regrowth tissue and determined the biomass. The ability to regrowth was then calculated as: % = biomass of regrowth tissue / biomass of removed tissue (Paudel et al., 2020).

To investigate the impact of herbivore cues on tolerance capacity in addition to artificial damage, plants with similar phenotypes (e.g., height, leaf number) were grouped in triad. Plants within the triad were randomly assigned to one of the three treatment: (1) undamaged control, (2) herbivore damage, (3) artificial damage. Herbivore damage was conducted by placing a single *M. sexta* larva (fifth instar) in a mesh cage that covered the entire plant and then allowed to feed freely for 24 h. Artificial damage was done based on the pattern of herbivore damage within each triad using dissecting scissors. Plants were kept under same water regime after 14 days, the percentage biomass of plant receiving damage treatments compared to undamaged control within the triad were calculated.

To investigate the impact of water availability on reproductive outputs after initial damage treatment, plants receiving the three damage treatments as described above were kept under the same water regime for 90 days. The flowering pattern was monitored daily throughout the period.

Flower bud differentiation was recorded for plants under 10 mL/day water treatment due to a lack of flower production. The numbers and biomass of fruits and seeds between treatments were also compared. The percentage of reproductive outputs of damaged plants compared to undamaged control plants was calculated as: % = counts of damaged plant/ counts of undamaged plant, for flowers, fruits, and seeds. Seeds were collected from individual fruits and pooled for each plant individual. Number of replicates for all experiments can be found in the relevant figure legends.

2.4. Resistance: caterpillar performance and defensive protein levels

To determine the overall resistance of tomato against caterpillars, three experiments were conducted: (1) on-plant relative growth rate (RGR) assay, (2) detached leaves RGR assay, (3) cunsumption assay.

For the on-plant RGR assay, a single larva (second instar, either

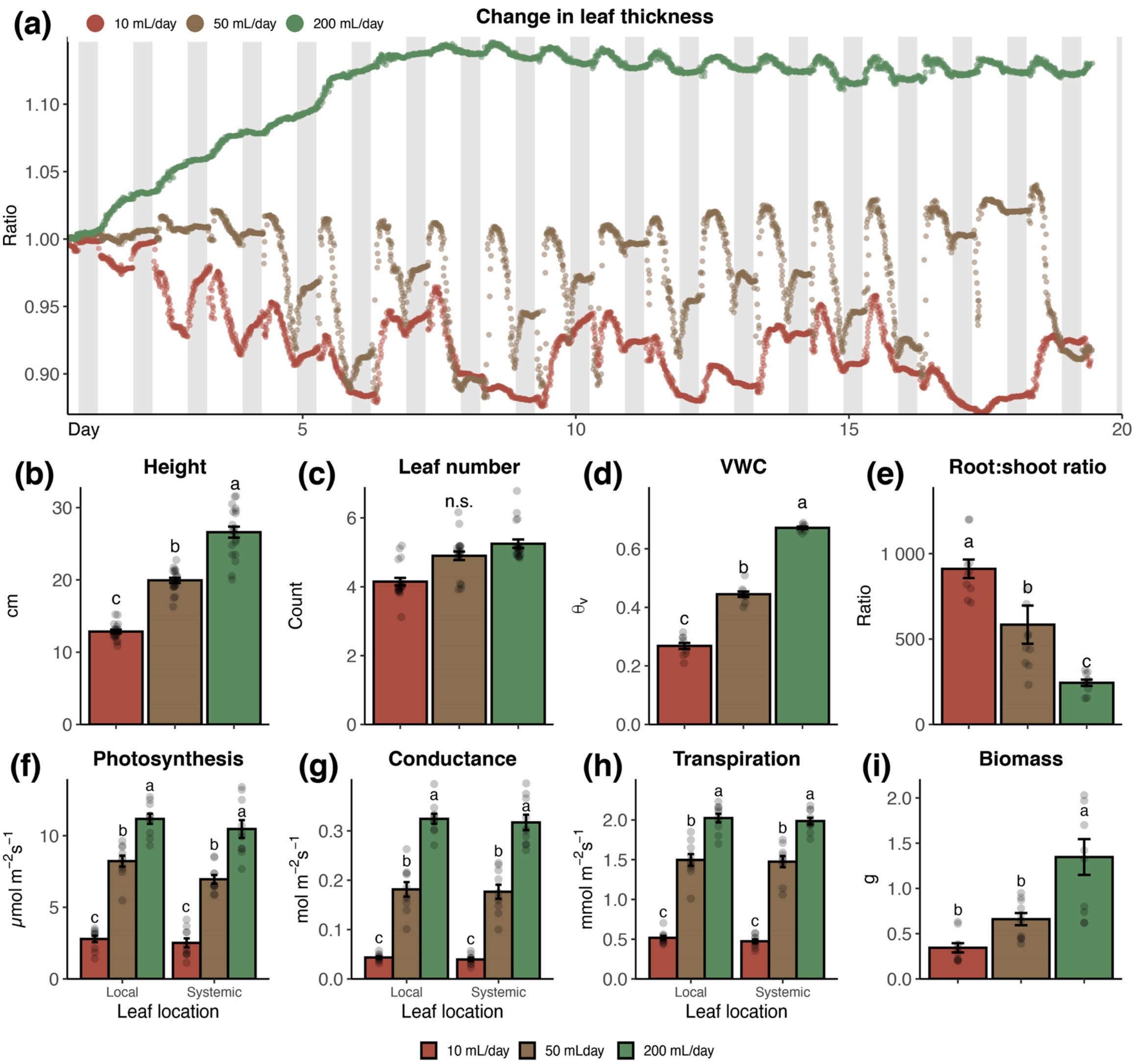

Fig. 1. Quantification of water status of tomato (*Solanum lycopersicum*) growing under different water availability. (a) leaf thickness fluctuation under the water regime. Dots indicate individual measurements per 10 min. Shaded area: dark period; non-shaded: light period. (b) Plant height from the cotyledon to the apical meristem (N = 10). (c) Number of mature leaves (N = 10, GLM, Poisson). (d) Soil volumetric water content (θ_v) (N = 10). (e) Root/shoot ratio (g/g) (N = 10). (f) Leaf photosynthetic activity (N = 10). (g) Leaf stomatal conductance (N = 10). (h) Leaf transpiration rate (N = 10). (i) Biomass of root and shoot (N = 10). Dots indicate individual observation. Values are the mean ± SE. Different letters indicate significant differences between means (ANOVA, Tukey HSD).

H. zea or *M. sexta*) was confined using mesh cages on the local leaf for 7 days. Because the mortality of *H. zea* is relatively high (25 %–85 %) on tomato, survival was recorded instead of the relative growth rate. For *M. sexta*, the relative growth rate (RGR) of larvae was calculated using the equation: (end weight-start weight) /((start weight + end weight)/ 2)*days) (Felton et al., 1989). Caterpillars were starved for 3 h to empty the alimentary canal before measuring their weights.

Since the water content in leaves also affects insect growth, an additional detached leaves RGR assay was performed to minimize the potential impact of fluctuating water content on RGR. Larvae of *M. sexta* were placed in 30 mL plastic cups with a layer of 1% agarose, which provides unlimited water source to caterpillars. Detached leaves from plants under different water treatments were collected daily, 3 h after water applications, for 7 days.

A consumption assay was conducted to determine suitability of plant under different water treatments to *M. sexta*. We estimated the amount of biomass removed by the caterpillar after 24 h by pairing plants with similar phenotypes together (same method as in 2.3), one of which received caterpillar treatment while the other served as undamaged controls. All plants were enclosed by a mesh cage. For damage treatment, a *M. sexta* larvae (fifth instar) was allowed to feed freely on a plant covered entirely with a mesh cage. To determine the amount of consumption, biomasses of the damaged plant and the undamaged control plant within pairs were measured. Consumption was calculated as: (biomass of damaged plant – biomass of undamaged plant)/ biomass of undamaged plant. In addition, the incidence of apical meristem removal was recorded.

The activity of two JA-regulated defensive proteins (Polyphenol oxidase, PPO, and Trypsin protease inhibitors, TPI) induced by both caterpillars were selected as markers of resistance traits. To induce the defensive protein, a single caterpillar was placed on the terminal leaflet of the local leaf inside a clip cage on a plant (Chung et al., 2013). Control plants had empty clip cages. Caterpillars (fifth instar *H. zea* and forth instar *M. sexta* larvae) were allowed to feed for 2 h to standardize the time and amount of damage. At 48 h post treatment, 50 mg leaf tissue from the terminal leaflet of both local (the leaflet damaged by the caterpillar) and systemic leaves were collected with liquid nitrogen and stored in -80 °C for further analysis.

The PPO activity was determined using a colorimetric assay that detects reaction of PPO with caffeic acid by changes in light absorbance at 450 nm. The TPI activity was determined using a colorimetric assay that detects inhibition of the reaction between trypsin and p-toluenesulfonyl-L-arginine methyl ester by changes in light absorbance at 247 nm. Inhibition index was calculated as: % inhibition/total protein (mg) in each reaction. Both methods were described in detail previously (Chung and Felton, 2011). Potential variations caused by water content of leaf was removed by standardization of protein activity with amount of plant protein in each reaction. Changes in primary metabolites were also investigated (Supplementary information M1). Number of replicates for all experiments can be found in the relevant figure legends.

2.5. *Statistical analyses*

All analyses were conducted using R version 3.6.3 (R Core Team, 2017). Continuous response variables, including plant height, VWC, root: shoot ratio, defensive protein level, RGR, plant biomass, flower and biomass percentages were fitted to general linear model and analyzed using ANOVA (package: *car*) (Fox et al., 2013). Diagnostic plots were performed to confirm the model assumptions, such as equal variance and normality, using package: *rcompanion* (Mangiafico, 2018) and function: *qqnorm* respectively (Zuur et al., 2010). If the assumptions were violated, the data were transformed using package: *bestNormalize* (Peterson, 2017). Tukey HSD *post-hoc* test (package: *lsmeans*) (Lenth, 2016) was used to test for differences between estimated marginal means.

Binary categorical responses, including insect survival and apical meristem damage, were analyzed using a generalized linear mixed model (GLMM) with binomial distribution (package: *lme4*) (Bates et al., 2014). Water treatment was set as a fixed factor in both models, whereas trial and insect weight were set as random factors. Count data, such as leaf number, were analyzed using generalized linear model (GLM) with Poisson distribution.

Data including total flower and total fruit number were analyzed using GLMM with package: *glmmTMB* (Brooks et al., 2017) to account for overdispersion and zero-inflation. Water and damage treatment were set as fixed factors, whereas apical meristem damage was set as a random factor. Total seed number, fruit percentage, and seed percentage were analyzed similarly but were fitted to GLMM with negative binomial distribution to account for overdispersion. All Diagnostic tests were ran using package: *DHARMa* (Hartig, 2017) to check for overdispersion and zero-inflation. Log-likelihood ratio test was performed to compare models fits.

3. Results

3.1. *Water status quantification*

Leaf thickness of the first mature leaf fluctuated with water availability (Fig. 1a). Leaf thickness remained relatively stable over the 14 days under high water availability. The daily increase of leaf thickness for all treatments indicated the time (9 a.m.) of water applications. The 50 mL/day and 10 mL/day treatments led to cessation of growth compared to 200 mL/day treatment over the 14 days indicated by a lack of increase in average leaf thickness. In 50 mL/day treatment, there was a sharp reduction in leaf thickness during the afternoon starting on the fourth day of water treatment. During the night, the leaf thickness in 50 mL/day treatment increased slightly. In 10 mL/day, leaf thickness in the afternoon started to decrease starting from the second day of water treatment, and the increase in leaf thickness during night ceased on the fourth day of the water treatment. The cessation of nighttime leaf thickness recovery is a distinct characteristic of 10 mL/day in comparison to 50 mL/day water treatment.

Daily pattern of leaf thickness fluctuation was caused by changes in light, temperature, and growth of leaf tissue. Lower water availability reduced plant height (Fig. 1b, $F_{2,57} = 183.49$, $P < 0.001$); did not affect leaf number (Fig. 1c); decreased soil volumetric water contents (Fig. 1d, $F_{2,26} = 551.31$, $P < 0.001$); but increased root to shoot ratio (Fig. 1e, $F_{2,27} = 21.09$, $P < 0.001$). Lower water availability decreased photosynthetic activity (Fig. 1f, $F_{2,56} = 229$, $P < 0.001$), stomatal conductance (Fig. 1g, $F_{2,56} = 114.7$, $P < 0.001$), transpiration rate (Fig. 1h, $F_{2.56} = 109.47$, $P < 0.001$) of local and systemic leaves. Lower water availability also reduced total biomass of plants (Fig. 1i, $F_{2,27} = 17.12$, $P < 0.001$).

3.2. *Tolerance under different water availability*

Compensatory regrowth following artificial damage was significantly reduced by lower water availability (Fig. 4a, $F_{2,59} = 34.08$, $P < 0.001$). Similar pattern was observed in experiment using single *M. sexta* larvae (fifth instar) (Fig. 4b). The percentage biomass (% = damaged/undamaged plant) 14 days after the damage was significantly reduced by lower water availability ($F_{2,26} = 29.83$, $P < 0.001$). There were no significant differences between the biomass removed from plant under different water availability.

Apical meristem damage significantly reduced percentage of flower (% = damaged/undamaged plant) regardless of water availability (Fig. 4c, $F_{1,41} = 10.33$, $P = 0.003$). Flower differentiation of plants grown under 10 mL/day water treatment was affected similarly by apical meristem damage (Fig. S2). Total flower number was negatively associated with damage treatment (Fig. 4d. GLMM, damage: A, estimate = 0.61, Z = -2.38, $P = 0.017$; damage: H, estimate = -0.51, Z = -1.95, $P = 0.052$), and positively associated with 200 mL/day water treatment when the plant was damaged (GLMM, interaction: 200 mL/day*A, estimate = 0.58, Z = 2.11, $P = 0.035$; interaction: 200 mL/day*H, estimate = 0.5, Z = 1.74, $P = 0.082$). Total fruit number was only positively associated with 200 mL/day water treatment (Fig. 4e. GLMM, water: 200 mL/day, estimate = 0.8, Z = 2.68, $P = 0.007$). Total seed number was negatively associated with damage by herbivores (Fig. 4f. GLMM, damage: H, estimate = -0.45, Z = -1.98, $P = 0.048$), and positively associated with 200 mL/day water treatment (GLMM, water: 200 mL/day, estimate = 0.46, Z = 2.33, $P = 0.020$). The percentage of flower (% = damaged/undamaged plant) was significantly reduced by lower water availability (Fig. 4g, $F_{1,41} = 10.33$, $P = 0.003$). Whereas, fruit and seed percentage were not affected by either damage or water treatment (Fig. 4h and Fig. 4i). The weight of fruit and seed showed a similar pattern (Fig. S3).

3.3. *Resistance under different water availability*

The mortality of *H. zea* larvae was not significantly affected by water availability (Fig. 3a). The RGR of *M. sexta* larvae was negatively affected by low water availability in both on-plant assay (Fig. 3b, $F_{2,81} = 5.66$, $P = 0.005$) and detached leaf assays (Fig. 3c, $F_{2,51} = 5.49$, $P = 0.007$). *M. sexta* larvae (fifth instar) consumed more leaf tissues on plant under 200 mL/day water treatment (Fig. 3d, $F_{2,42} = 9.41$, $P < 0.001$). However, the percentage of plant tissue removed by *M. sexta* were higher in plants under 10 mL/day treatment (Fig. 3e, $F_{2,42} = 16.1$, $P < 0.001$). The apical meristems of plants under 10 mL/day water treatment were also more likely to be damaged by caterpillars (Fig. 3f, GLMM, wtr: 50 mL/day, estimate = -2.08, Z = -2.46, $P = 0.014$; wtr: 200 mL/day, estimate = -2.4, Z = -2.76, $P = 0.006$).

Lower water availability increased the levels of constitutive and induced defensive proteins in leaves. The induction pattern was similar between *H. zea* and *M. sexta*. For *H. zea*, lower water availability and damage increased the PPO activity (Fig. 4a, water: $F_{2,124} = 11.19$, $P < 0.001$; damage: $F_{1,124} = 4.98$, $P = 0.027$). Systemic leaves had higher PPO activity ($F_{2,124} = 5.03$, $P = 0.027$). The effect of damage was only significant in local leaf indicated by a significant interaction between leaf location and damage ($F_{1,124} = 5.73$, $P = 0.018$). Similarly, lower water availability and damage by *H. zea* increased the level of TPI (Fig. 4b, water: $F_{2,120} = 46.54$, $P < 0.001$; damage: $F_{1,120} = 87.49$, $P < 0.001$). Local leaves had higher level of TPI ($F_{1,120} = 37.03$, $P < 0.001$). The effect of damage was only significant in local leaf indicated by a

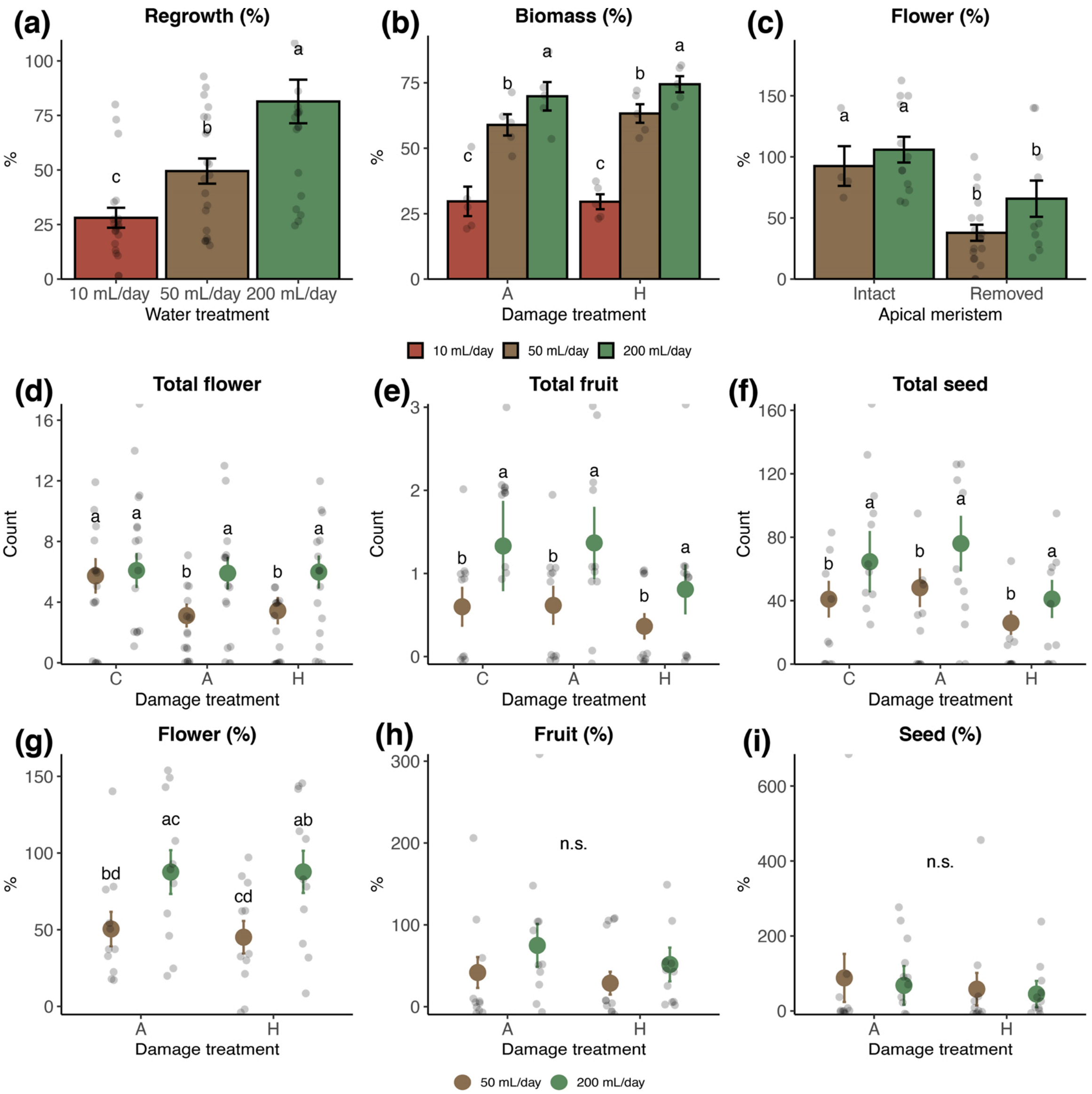

Fig. 2. Impact of water availability on tolerance of tomato against herbivory. (a) Percentage of regrowth 10 days after artificially removed same amount of shoot (N = 21). (b) Percentage of biomass remained compared to undamaged control 14 days after damaged by *Manduca sexta*. (c). Impact of apical meristem damage on percentage of flower produced after damage compared to undamaged control. (d) Total number of flowers. (e) Total number of fruits. (f) Total number of seeds. (g) Percentage of flower (% = damaged/undamaged plant). (h) Percentage of fruit (% = damaged/undamaged plant). (i) Percentage of seed (% = damaged/undamaged plant). C: undamaged control; A: artificial damage; H: herbivore damage. N = 16 for (b)-(i). Dots indicate individual observation. Values represent the means ± SE. Different letters indicate significant differences between means based on the fitted model (Tukey HSD).

significant interaction between location and damage ($F_{1,120} = 32.95$, $P < 0.001$). For *M. sexta*, lower water availability and damage increased the PPO activity (Fig. 4c, water: $F_{2,100} = 24.33$, $P < 0.001$; damage: $F_{1,100} = 161.54$, $P < 0.001$). Local leaves had higher PPO activity ($F_{1,100} = 260.43$, $P < 0.001$). The effect of damage was only significant in local leaf indicated by a significant interaction between location and damage ($F_{1,100} = 161.2$, $P < 0.001$). In addition, the effect of water availability was only significant in local leaf indicated by a significant interaction between location and damage ($F_{1,100} = 6.94$, $P = 0.002$). Similarly, lower water availability and damage by *M. sexta* increased the level of TPI (Fig. 4d, water: $F_{2,114} = 18.57$, $P < 0.001$; damage: $F_{1,114} = 78.25$, $P < 0.001$). Local leaves had higher level of TPI ($F_{1,114} = 64.82$, $P < 0.001$). The effect of damage was only significant in local leaves indicated by a significant interaction between location and damage ($F_{1,114} = 52.37$, $P < 0.001$). Similar patterns were observed in an independent experiment (Fig. S4). We found that all concentrations of primary metabolites increased in plants under lower water availability (Fig. S5, Supplementary information R1).

4. Discussion

4.1. Tolerance and water availability

Tolerance to herbivory is a vital strategy of plant to cope with herbivory, and the expression of which is mediated by the availability of critical resources in the environments (Wise and Abrahamson, 2007). Our findings reveal an unexpected case where tolerance became lower under water limitation. The expression of tolerance is associated with many plant characteristics that are affected by resource availability, including growth rate (Gianoli and Salgado-Luarte, 2017), nutrient uptake ability (Ruess et al., 1983), photosynthesis activity (Turnbull et al., 2007), storage organ (Eyles et al., 2009), and architecture (Mcnaughton, 1983). The Limiting Resource Model is currently the best model that predicts the direction and intensity of impacts that resources

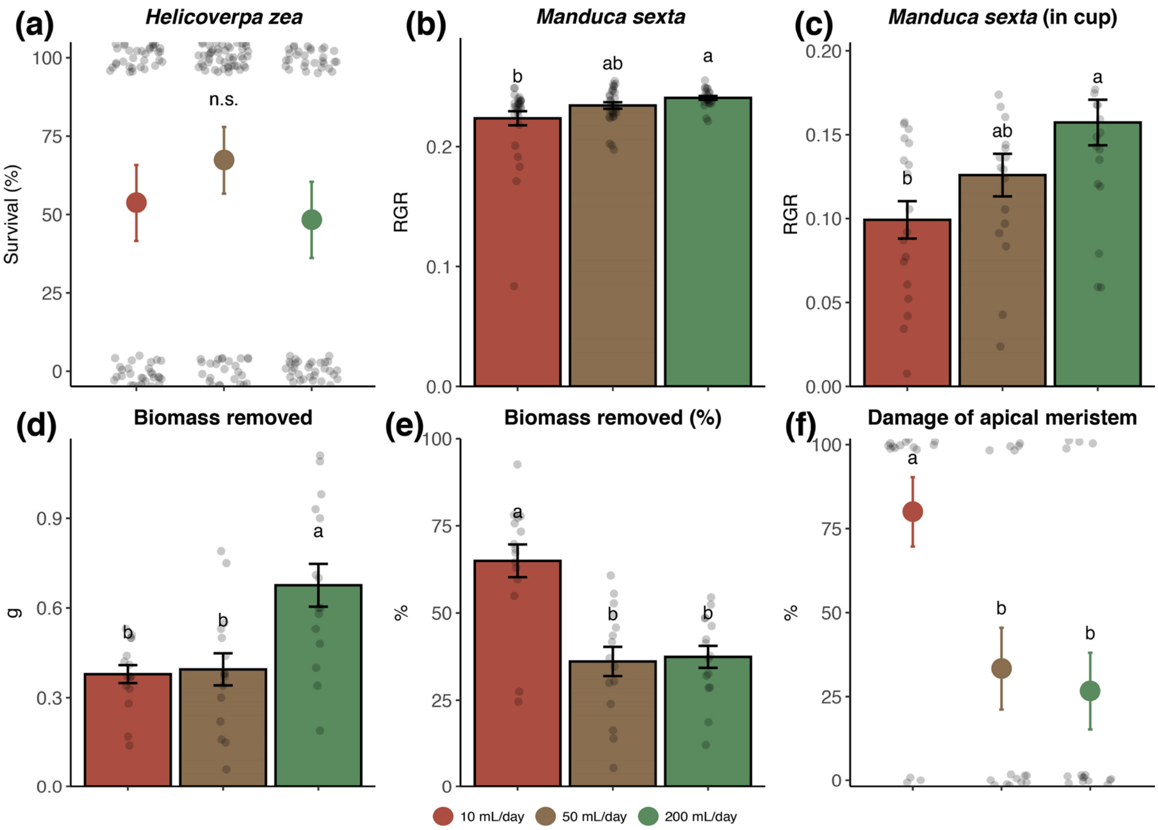

Fig. 3. Performance of caterpillars feeding on plants under different water availability. (a) Survival of *Helicoverpa zea* on tomato (N = 60). (b) Relative growth rate (RGR) of *Manduca sexta* on tomato (N = 30). (c) RGR of *M. sexta* in cup assay (N = 20). (d) Biomass removed by *M. sexta.* (e) Percentage of biomass removed by *M. sexta* (N = 16). (f) Probability of apical meristem damaged by *M. sexta* (N = 16). Dots indicate individual observation. Values are the mean ± SE. Different letter indicates significant differences between means based on the fitted model (Tukey HSD).

have on plant tolerance to herbivory, and can predicts 95 % of outcomes in the literature (Wise and Abrahamson, 2007). Although the studies that investigate the impacts of water availability on tolerance remain scarce, it is predicted by LRM that plants growing under low water availability should have a higher tolerance to leaf herbivory. This prediction was supported in a few systems, including soybean *Glycine max* (Shimada et al., 1992), rapeseed *Brassica napus* (Nowatzki and Weiss, 1997), and pigweed *Amaranthus hybridus* (Gassmann, 2004). However, we find a complete opposite pattern where plants under low water availability had lower tolerance to herbivory (see (Atala and Gianoli, 2009)). This conclusion was based on lower (1) compensatory regrowth (Fig. 2a), (2) percentage of biomass (Fig. 2b), and (3) flower production (Fig. 2g) under lower water availability. A closer investigation reveals that studies by both Shimada et al. (1992) and Nowatzki and Weiss (1997) did not use a ratio based tolerance indices (Strauss and Agrawal, 1999) that compares between damaged and undamaged plants. Shimada et al. (1992) concluded a higher tolerance of soybean under low water availability following defoliation based on observed increase in several physiological traits, including photosynthesis, stomatal conductance, water potential, and grain yield. However, higher compensatory photosynthesis does not guarantee a higher tolerance. For example, higher root to shoot ratio but not the photosynthesis activity, explained more of the higher tolerance of *A. hybridus* under low water availability (Gassmann, 2004). In contrast, higher root to shoot ratio in tomato growing under low water availability was not linked to higher tolerance in our study.

Variation in tolerance among systems could be influenced by biology of the plant as well as the parameterization of tolerance. While compensatory photosynthesis has been proposed as a major mechanism that facilitates tolerance, the immediate upregulation of photosynthetic activity and stomatal conductance of the remaining leaves can be a functional response to the reduction of respiration after defoliation, and therefore a release from water limitation, rather than an adaptive response to herbivory (Mcnaughton, 1983). The observation that the tolerance parameters, such as regrowth capacity and flower number were negatively affected by low water availability but not fruit or seed, reveals the complexity of tolerance expression and the discrepancy among tolerant parameters or methodology. This finding strongly suggested the need for multiple parameters to provide a more comprehensive view on plant tolerance to herbivory. In addition, we discover a strong association between apical damage and a reduction in flower number. The finding is quite surprising since apical damage was required for expression of tolerance in some plants (Aarssen and Irwin, 1991). However, the reproductive success of tomato plants seems to depend on an intact apical meristem, and low water availability increases the risk of apical damage by herbivores. Consequently, the damage pattern by herbivores adds another layer of complexity to the expression of tolerance and suggest a need to use ecological relevant damage treatments in study of plant tolerance.

4.2. Tolerance and resistance

The simultaneous increase in resistance and decrease in tolerance of tomato under water limitation suggest a potential physiological tradeoff between the two protection strategies against herbivore. The evolution of tolerance is affected not only by herbivory but also likely by other factors such as plant resistance to herbivores (Fornoni, 2011). The potential redundancy between tolerance and resistance in reducing fitness cost of herbivory has led to many studies investigating the evolutionary tradeoff of these two strategies (Núñez-Farfán et al., 2007), and also the

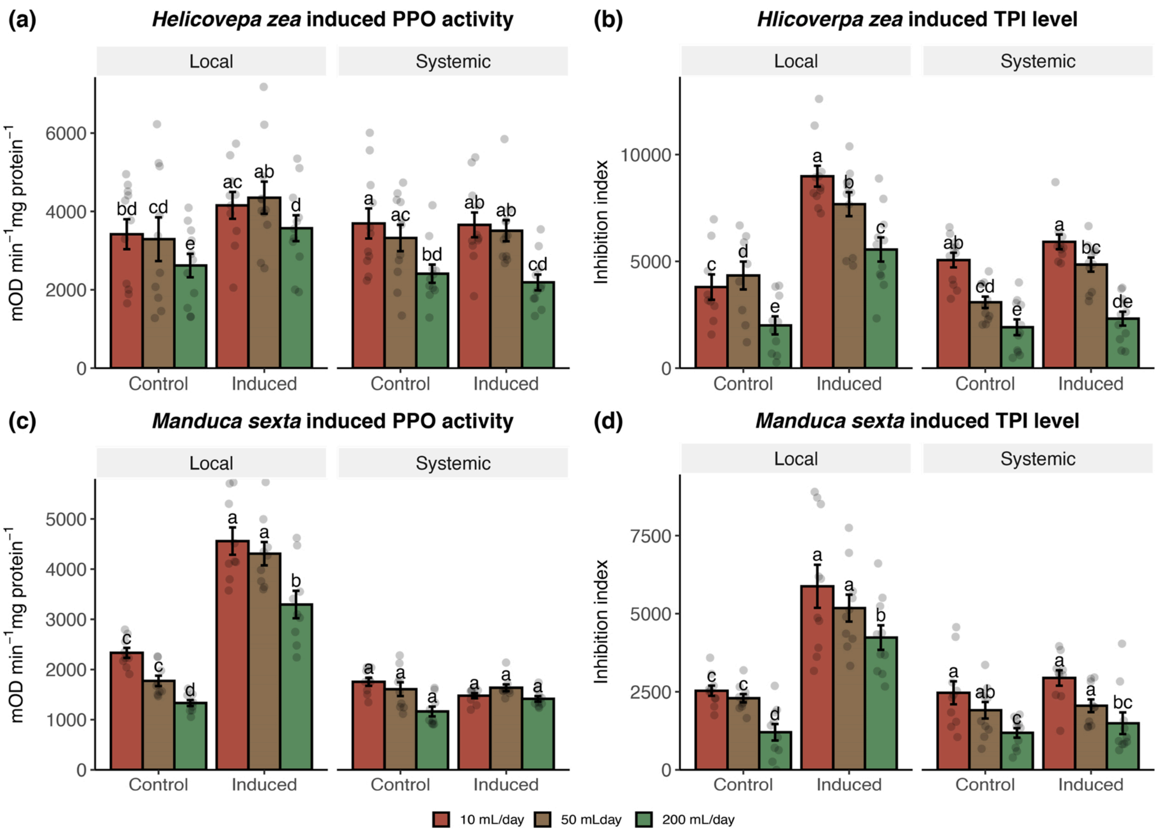

Fig. 4. Changes in level of constitutive, induced defensive proteins in leaves of tomato growing under different water availability. (a) PPO activity induced by *Helicoverpa zea* (N = 11). (b) TPI activity induced by *H. zea* (N = 11). (c) PPO activity induced by *Manduca sexta* (N = 9). (d) TPI activity induced by *M. sexta* (N = 10). Values indicate the mean ± SE. Dots indicate individual observation. Different letters indicate significant differences between means within each location (Tukey HSD, ANOVA).

discovery that evolutionary tradeoffs between tolerance and resistance are linked to resource availability. For example, species in habitat with higher resource availability have higher tolerance and lower resistance, and vice versa (see Coley et al., 1985). Resource availability was also used to predict the patterns of growth and defense in plants. The growth-differentiation balance (GDB) framework predicts a physiological tradeoff between growth/competitive ability and differentiating processes (Herms and Mattson, 1992). Specifically, the GDB framework predicts that environmental factors, including water stress, that limit growth more than photosynthesis (i.e., create a carbon sink limited

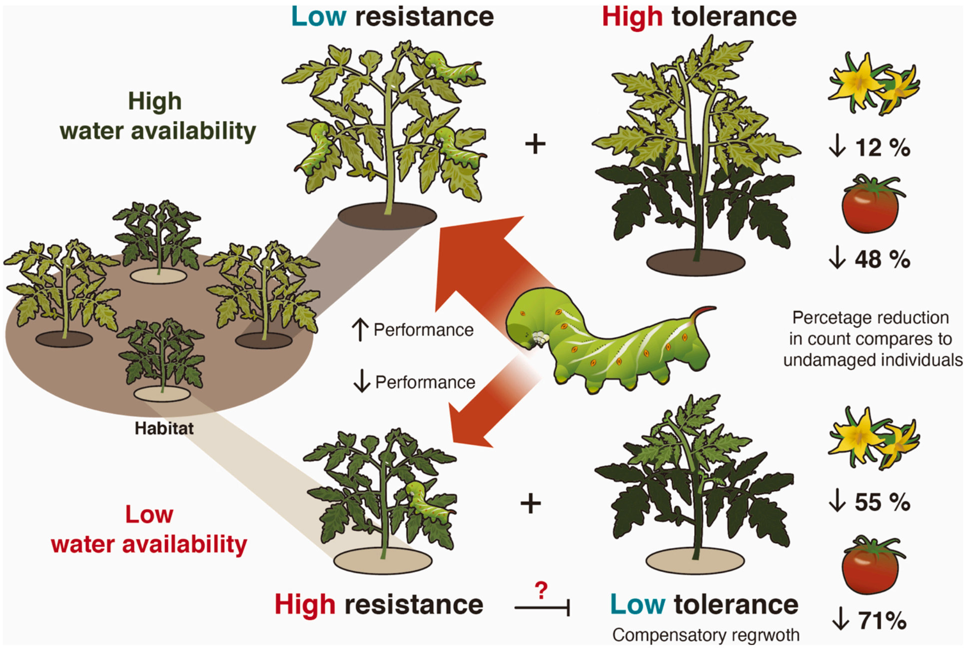

Fig. 5. Graphical summary. Low water availability increases the resistance of tomato against herbivore indicated by a lower performance of herbivore. Low water availability reduces the regrowth ability and flowers (% = damaged/undamaged plant) after herbivory. Fruits (% = damaged/undamaged plant) shows a similar reduction but was not statistically significant. The results show an increase in resistance and decrease in tolerance of tomato to herbivory under low water availability, suggesting a potential tradeoff between the two strategies.

plant) increase the resource pool available for resistance traits, such as carbon-based secondary metabolites (Herms and Mattson, 1992). The observation that water limitation simultaneous increases resistance and decreases tolerance of tomato supports this prediction. Water limitation increases the constitutive and induced defenses in tomato. The increase in resistance was confirmed by reduced herbivore performance and consumption. From a plant perspective, resistance traits might be important under water limitation based on the finding that similar herbivore stress (i.e., a *M. sexta* larvae) caused more percentage damage to the plants, suggesting a higher cost of herbivory. We speculate that low water availability might promote the selection of phenotypes (i.e., higher resistance) that prevent tissue loss to ensure certain level of competitive ability which is constrained by low water availability due to a reduction in growth and tolerance (Siemens et al., 2002). Although the resistance traits investigated in this study are essentially nitrogen-based, similar principles might apply where an excessive nitrogen under low water availability (Wang et al., 2003) is reallocated to differentiating traits, such as defense proteins. Although whether this physiological tradeoff is adaptive remain unknown, we hypothesize that increase in resistance under low water availability might be a common strategy of herbaceous plants that constantly experience variations in water availability in natural habitats, according to the location of individual plants determined by their dispersal strategies (Núñez-Farfán et al., 2007).

4.3. Conclusion

As the importance of drought increased in the future due to climate change, understanding the changes in tolerance under water limitation in addition to resistance is crucial in unraveling the complex physiological and ecological interactions between plants, insect herbivores, and their environment. Our study reveals an unexpected reduction in tolerance of tomato to herbivory and a concomitant increase in resistance of tomato to a specialist herbivore under low water availability (Fig. 5), suggesting a physiological tradeoff between the two strategies. The finding that tolerance capacity is influenced by apical meristem damages shows the importance of damage pattern on the expression of tolerance and the differences in damage cost associated with distinct tissues. This study contributes to the limited but growing knowledge on how water availability affects tolerance of plant to herbivory.

Declaration of authorship

PAL and GWF conceived the ideas, designed experiments, and analyzed the data. PAL, SP, AA and NLS performed the experiment. PAL, SP, and GWF wrote the manuscript.

Declaration of Competing Interest

The authors declare no competing interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2020.104334.

References

Aarssen, L.W., Irwin, D.L., 1991. What selection: herbivory or competition? Oikos 60, 261–262.

Acevedo, F.E., Rivera-Vega, L.J., Chung, S.H., Ray, S., Felton, G.W., 2015. Cues from chewing insects - the intersection of DAMPs, HAMPs, MAMPs and effectors. Curr. Opin. Plant Biol. 26, 80–86.

Afzal, A., Duiker, S.W., Watson, J.E., Luthe, D., 2017. Leaf thickness and electrical capacitance as measures of plant water status. T. ASABE 60, 1063–1074.

Atala, C., Gianoli, E., 2009. Effect of water availability on tolerance of leaf damage in tall morning glory, *Ipomoea purpurea*. Acta Oecol. Montrouge (Montrouge) 35, 236–242.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting linear mixed-effects models using lme4. arXiv preprint 1406 (5823).

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Machler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9, 378–400.

Chung, S.H., Felton, G.W., 2011. Specificity of induced resistance in tomato against specialist lepidopteran and coleopteran species. J. Chem. Ecol. 37, 378–386.

Chung, S.H., Rosa, C., Scully, E.D., Peiffer, M., Tooker, J.F., Hoover, K., Luthe, D.S., Felton, G.W., 2013. Herbivore exploits orally secreted bacteria to suppress plant defenses. Proc. Natl. Acad. Sci. U. S. A. 110, 15728–15733.

Coley, P.D., Bryant, J.P., Chapin 3rd, F.S., 1985. Resource availability and plant antiherbivore defense. Science 230, 895–899.

Dixon, A.F., Kindlmann, P., 1990. Role of plant abundance in determining the abundance of herbivorous insects. Oecologia 83, 281–283.

Eyles, A., Pinkard, E.A., Mohammed, C., 2009. Shifts in biomass and resource allocation patterns following defoliation in *Eucalyptus globulus* growing with varying water and nutrient supplies. Tree Physiol. 29, 753–764.

Felton, G.W., Donato, K., Del Vecchio, R.J., Duffey, S.S., 1989. Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. J. Chem. Ecol. 15, 2667–2694.

Fornoni, J., 2011. Ecological and evolutionary implications of plant tolerance to herbivory. Funct. Ecol. 25, 399–407.

Fox, J., Friendly, M., Weisberg, S., 2013. Hypothesis tests for multivariate linear models using the car package. R J. 5, 39–52.

Gassmann, A.J., 2004. Effect of photosynthetic efficiency and water availability on tolerance of leaf removal in *Amaranthus hybridus*. J. Ecol. 92, 882–892.

Gatehouse, J.A., 2002. Plant resistance towards insect herbivores: a dynamic interaction. New Phytol. 156, 145–169.

Gianoli, E., Salgado-Luarte, C., 2017. Tolerance to herbivory and the resource availability hypothesis. Biol. Lett. 13, 20170120.

Hartig, F., 2017. DHARMa: Residual Diagnostics for Hierarchical (multi-level/mixed) Regression Models. R Package Version 0.1 5, p. 5.

Herms, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or defend. Q. Rev. Biol. 67, 283–335.

Howe, G.A., Jander, G., 2008. Plant immunity to insect herbivores. Annu. Rev. Plant Biol. 59, 41–66.

Huberty, A.F., Denno, R.F., 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. Ecology 85, 1383–1398.

Jamieson, M.A., Trowbridge, A.M., Raffa, K.F., Lindroth, R.L., 2012. Consequences of climate warming and altered precipitation patterns for plant-insect and multitrophic interactions. Plant Physiol. 160, 1719–1727.

Jones, H.G., 2007. Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. J. Exp. Bot. 58, 119–130.

Lenth, R.V., 2016. Least-squares means: the R package lsmeans. J. Stat. Softw. 69, 1–33.

Mangiafico, S., 2018. Rcompanion: Functions to Support Extension Education Program Evaluation. R Package Version 2.0. 0.

Maron, J.L., Crone, E., 2006. Herbivory: effects on plant abundance, distribution and population growth. P. Roy. Soc. B-Biol. Sci. 273, 2575–2584.

Mattson, W.J., Haack, R.A., 1987. The role of drought in outbreaks of plant-eating insects. Bioscience 37, 110–118.

Mcnaughton, S.J., 1983. Compensatory plant-growth as a response to herbivory. Oikos 40, 329–336.

National Research Council, 2011. Advancing the Science of Climate Change. National Academies Press.

Nowatzki, T.M., Weiss, M.J., 1997. Effects of simulated and flea beetle injury to cotyledons on growth of drought-stressed oilseed rape, *Brassica napus* L. Can. J. Plant Sci. 77, 475–481.

Núñez-Farfán, J., Fornoni, J., Valverde, P.L., 2007. The evolution of resistance and tolerance to herbivores. Annu. Rev. Ecol. Evol. Syst. 38, 541–566.

Orians, C.M., Schweiger, R., Dukes, J.S., Scott, E.R., Müller, C., 2019. Combined impacts of prolonged drought and warming on plant size and foliar chemistry. Ann. Bot.

Paudel, S., Lin, P.-A., Hoover, K., Felton, G.W., Rajotte, E.G., 2020. Asymmetric responses to climate change: temperature differentially alters herbivore salivary elicitor and host plant responses to herbivory. J. Chem. Ecol.

Peiffer, M., Felton, G.W., 2009. Do caterpillars secrete "oral secretions"? J. Chem. Ecol. 35, 326–335.

Pena-Cortes, H., Fisahn, J., Willmitzer, L., 1995. Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. Proc. Natl. Acad. Sci. U. S. A. 92, 4106–4113.

Peterson, R., 2017. Estimating Normalization Transformations With bestNormalize.

Pineda, A., Pangesti, N., Soler, R., van Dam, N.M., van Loon, J.J., Dicke, M., 2016. Negative impact of drought stress on a generalist leaf chewer and a phloem feeder is associated with, but not explained by an increase in herbivore-induced indole glucosinolates. Environ. Exp. Bot. 123, 88–97.

Price, P.W., 1991. The plant vigor hypothesis and herbivore attack. Oikos 62, 244–251.

R Core Team, 2017. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ruess, R.W., McNaughton, S.J., Coughenour, M.B., 1983. The effects of clipping, nitrogen source and nitrogen concentration on the growth responses and nitrogen uptake of an east african sedge. Oecologia 59, 253–261.

Shimada, S., Kokubun, M., Shibata, H., Matsui, S., 1992. Effect of water supply and defoliation on photosynthesis, transpiration and yield of soybean. Japan. J. Crop. Sci. 61, 264–270.

Siemens, D.H., Garner, S.H., Mitchell-Olds, T., Callaway, R.M., 2002. Cost of defense in the context of plant competition: *brassica rapa* may grow and defend. Ecology 83, 505–517.

Strauss, S.Y., Agrawal, A.A., 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14, 179–185.

Sun, Y., Ding, J., Frye, M.J., 2010. Effects of resource availability on tolerance of herbivory in the invasive *Alternanthera philoxeroides* and the native *Alternanthera sessilis*. Weed Res. 50, 527–536.

Tian, D., Peiffer, M., Shoemaker, E., Tooker, J., Haubruge, E., Francis, F., Luthe, D.S., Felton, G.W., 2012. Salivary glucose oxidase from caterpillars mediates the induction of rapid and delayed-induced defenses in the tomato plant. PLoS One 7, e36168.

Tian, D., Peiffer, M., De Moraes, C.M., Felton, G.W., 2014. Roles of ethylene and jasmonic acid in systemic induced defense in tomato (*Solanum lycopersicum*) against Helicoverpa zea. Planta 239, 577–589.

Turnbull, T.L., Adams, M.A., Warren, C.R., 2007. Increased photosynthesis following partial defoliation of field-grown *Eucalyptus globulus* seedlings is not caused by increased leaf nitrogen. Tree Physiol. 27, 1481–1492.

Wang, W., Vinocur, B., Altman, A., 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218, 1–14.

Waring, G.L., Cobb, N.S., 1992. The impact of plant stress on herbivore population dynamics. Insect-Plant Interactions 4, 167–226.

White, T.C., 1974. A hypothesis to explain outbreaks of looper caterpillars, with special reference to populations of Selidosema suavis in a plantation of *Pinus radiata* in New Zealand. Oecologia 16, 279–301.

Wise, M.J., Abrahamson, W.G., 2007. Effects of resource availability on tolerance of herbivory: a review and assessment of three opposing models. Am. Nat. 169, 443–454.

Wolfson, J.L., Murdock, L.L., 1990. Growth of *Manduca sexta* on wounded tomato plants - role of induced proteinase inhibitors. Entomol. Exp. Appl. 54, 257–264.

Xie, H., Shi, J., Shi, F., Xu, H., He, K., Wang, Z., 2020. Aphid fecundity and aphid defences in wheat exposed to a combination of heat and drought stresses. J. Exp. Bot.

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14.