Scott ER, Li X, Kfoury N, Morimoto J, Han W-Yan, Ahmed S, Cash SB, Griffin TS, Stepp JR, Robbat A, Orians CM, Interactive effects of drought severity and simulated herbivory on tea (Camellia sinensis) volatile and non-volatile metabolites, *Environmental and Experimental Botany* 157 (January 2019): 283–292. doi:10.1016/j.envexpbot.2018.10.025.

Interactive effects of drought severity and simulated herbivory on tea (*Camellia sinensis*) volatile and non-volatile metabolites.

Eric R. Scott*[a], Xin Li[b], Nicole Kfoury[c, d], Joshua Morimoto[c], Wen-Yan Han[b], Selena Ahmed[e], Sean B. Cash[d, f], Timothy S. Griffin[f], John R. Stepp[g], Albert Robbat Jr.[c, d], Colin M. Orians[a, d]

[a] Department of Biology, Tufts University, Medford, MA 02155, USA

[b] Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China

[c] Department of Chemistry, Tufts University, Medford, MA 02155, USA

[d] Tufts University Sensory and Science Center, Medford, MA 02155, USA

[e] Department of Health and Human Development, Montana State University, Bozeman, MT 59717, USA

[f] Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA, USA

[g] Department of Anthropology, University of Florida, Gainesville, FL 32611, USA

* Corresponding author: eric.scott@tufts.edu

Highlights

- We simulated herbivory on moderately and severely drought stressed tea plants.
- Non-volatile metabolites were unaffected by drought stress nor simulated herbivory.
- Most volatiles were not induced by simulated herbivory under severe drought.
- Methyl salicylate had greater induction by simulated herbivory under severe drought.
- Drought affects plant responses to biotic stress with consequences for tea quality.

Abstract

Plants often experience multiple sources of stress simultaneously, yet little is known about interactive effects of multiple stressors on plant metabolic responses. Plants are well known to respond to both drought and insect herbivory through the induced production of secondary metabolites. However, severe drought stress limits photosynthesis and may therefore inhibit the production of induced secondary metabolites in response to herbivory due to carbon limitation. On the other hand, drought-stressed plants may be primed to respond more strongly to herbivory due to hormonal crosstalk or redundancy of metabolites that are produced in response to drought and herbivory. We tested the interactive effects of drought and simulated herbivory in tea plants (*Camellia sinensis* (L.) Kuntze var. *sinensis*) grown in the field under varying rainfall interception treatments and then exposed to an exogenous methyl jasmonate (MeJA) treatment. We show that severe drought generally inhibits the induction of secondary metabolites by exogenous MeJA (simulated herbivory). However, a few volatile metabolites, including methyl salicylate, are more strongly induced by MeJA in severely drought-stressed plants compared to moderately stressed plants, possibly due to priming by drought stress. Our approach of using multiple levels of drought stress and a targeted/untargeted approach to measuring volatile metabolites was essential to discovering these patterns of induction. In addition to having implications for plant-herbivore interactions in the presence of abiotic stress, these results have important implications for tea quality.

Abbreviations

MeJA, methyl jasmonate; JA, jasmonic acid; SA, salicylic acid; BACE, Boston Area Climate Experiment; GC/MS, gas chromatography/mass spectrometry; LC/MS, liquid chromatography/mass spectrometry; PLS-DA, partial least squares discriminant analysis; VIP, variable importance in projection; RPA, relative peak area.

Keywords

Drought stress, methyl jasmonate, multiple stressors, plant-insect interactions, plant volatiles, tea (*Camellia sinensis*).

1. Introduction

Plants in their natural environment often experience sequential and multiple stresses that can reduce fitness. Both abiotic and biotic stresses can reduce plant growth and reproduction and can potentially cause death. Plants respond to abiotic stresses such as drought with dramatic changes in primary and secondary metabolism (Niinemets, 2015). Some studies have shown that drought stress increases concentrations of secondary metabolites, many of which act as osmolytes that prevent water loss and/or antioxidants to reduce oxidative stress (Cao et al., 2007; Reddy et al., 2004; Zobayed et al., 2007). To the contrary, other studies have found no effect of drought on secondary metabolites (Dunford and Vazquez, 2005; Nogués et al., 2015), or a decrease in secondary metabolites (Marchese et al., 2010; Yani et al., 1993). The observed variation in metabolite responses to drought in the literature may be due to yet-unexplored ecological factors, or researchers may not be capturing the full range of metabolite responses to drought by focusing

only on a subset of metabolites or only including a single level of drought stress in their drought treatments.

Insect herbivory is one of the most important sources of biotic stress for plants. Insect herbivory can also reduce fitness, and plants have evolved to produce induced secondary metabolites in response to herbivory that act as direct or indirect defenses (Bennett and Wallsgrove, 1994; Cipollini, 2010; Karban et al., 1997). Induced secondary metabolites can include both volatile and non-volatile chemicals with a wide range of structures and modes of action. The biochemical pathways involved in defense induction in plants are well resolved—chewing herbivores typically activate the jasmonic acid (JA) signaling pathway and phloem feeders often activate the salicylic acid (SA) signaling pathway (Lortzing and Steppuhn, 2016).

Plant responses to biotic and abiotic stresses are often studied by exposing plants to one stress at a time. If plant responses to multiple stresses are additive, such studies are valid for predicting responses to combined stresses. For example, additive, opposite effects of drought and insect herbivory on secondary metabolites were observed in a study on *Arabidopsis thaliana* (Mewis et al., 2012). Aliphatic glucosinolate and flavonoid concentrations were increased by water stress and decreased by aphid feeding, but when plants experienced both sources of stress, aliphatic glucosinolate concentrations were similar to those of control plants. However, exposure to multiple environmental stressors may also have interactive effects on secondary metabolites due to shared metabolic and hormonal pathways. Plant hormones that play a role in herbivore defense responses (JA and SA) also play a role in plant drought responses (Munné-Bosch and Peñuelas, 2003; Riemann et al., 2015; Weldegergis et al., 2018, 2015). The multiple roles of

plant hormones can lead to interactive responses to herbivory and drought stress. For example, methyl salicylate (an SA derivative) showed an interactive response to drought and herbivory in *Alnus glutinosa,* which resulted in faster herbivore-induced emission of (*E*)-β-ocimene and (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) in drought-stressed plants compared to plants experiencing drought or herbivory alone (Copolovici et al., 2014).

Interactive effects might also emerge as a result of plants allocating limited resources to respond to multiple stresses. Previous research has shown that drought stress can reduce constitutive levels of secondary metabolites in plants. For example, Pegoraro et al. (2004) found reduction of isoprene emissions in oak trees during drought stress, and hypothesized this was due to carbon limitation caused by a concomitant reduction in photosynthetic rate. Marchese et al. (2010) found a decrease in the terpene artemisinin in *Artemesia annua* under severe drought stress, although moderate drought stress resulted in an increase in artemisinin concentration. This was also interpreted by the authors to be due to carbon limitation imposed by impaired photosynthesis under severe drought stress, but not under moderate drought stress. If the reduction in constitutive secondary metabolites under drought stress is due to carbon limitation imposed by impaired photosynthesis, we might also expect the magnitude of induction of secondary metabolites to follow the same pattern. That is, the "inducibility" of secondary metabolites should be reduced in severely drought-stressed plants compared to moderately drought-stressed or unstressed plants.

Tea (*Camellia sinensis*) is an excellent study system as the plants produce structurally and functionally diverse volatile and non-volatile metabolites, the concentrations of which are often determined by abiotic and biotic factors including drought and herbivory (Ahmed et al., 2014b;

Cai et al., 2013; Han et al., 2017; Kfoury et al., 2018b). Moreover, these metabolites are important determinants of sensory quality and health benefits of brewed tea (Camfield et al., 2014; Dufresne and Farnworth, 2001). Furthermore, no study, to our knowledge, investigates interactive effects of drought and herbivory on tea metabolites in the field. In this study, we exposed tea plants to three precipitation treatments—ambient (100%), 75% of ambient, and 50% of ambient—followed by a methyl jasmonate (MeJA) treatment (to simulate herbivory) to determine if induced responses to herbivory depend on the severity of water limitation. We predict that drought stress will reduce photosynthetic activity, which could inhibit metabolite induction by simulated herbivory due to carbon limitation on *de novo* production of secondary metabolites. However, drought stress could also lead to priming of anti-herbivore defenses due to hormonal cross-talk, which would result in increased production of herbivore-induced compounds after MeJA treatment.

2. Materials and Methods

2.1 Plants

Three- to four-year-old tea plants (*Camellia sinensis* var. *sinensis*) were purchased from Logee's Nursery (Danielson, CT) and repotted into 11.4 cm deep pots using soil from the BACE site (see below) that was sifted and mixed with 50% peat moss to increase soil acidity, since tea is an acid-loving plant. Plants were initially covered with shade cloth to help them adjust to outdoor conditions and watered regularly until established. Tea plants were free of visible herbivore damage and pathogen attack throughout the experiment.

2.2 Experimental Design

This experiment was conducted at the Boston Area Climate Experiment (BACE), described in Hoeppner and Dukes (2012) (Fig. S1), between June 18 and August 25, 2017. We used the un-warmed plots from BACE under modified precipitation treatments, namely, 75% of ambient rainfall, and 50% of ambient rainfall. Reduced precipitation treatments were produced by adjusting the spacing of clear, corrugated polycarbonate slats covering the plots. Ambient rainfall treatment plots were covered with deer fencing to reduce photosynthetically active radiation by about 5%, which approximates light interception by the polycarbonate slats in the drought treatments (Auyeung et al., 2013; Hoeppner and Dukes, 2012). The polycarbonate slats reduce UV-A and UV-B radiation by ~25% and ~50% at mid-day, respectively (data not shown). Since blocking nearly 100% of UV has no effect on most phenolics (Strømme et al., 2018), we did not expect the UV differences between plots to interfere with the effects of drought on phenolic compounds of tea. These three precipitation treatments were replicated three times for a total of nine plots. Experimental plots were prepared by removing vegetation from a 1 m x 2 m area in each plot, which were surrounded by hardware cloth barriers (about 46 cm high, about 13 cm below soil) to exclude rodent herbivores.

Starting on June 18, 10 tea plants were planted into the ground (in pots) in each plot for a total of 90 plants. Planting was done in three batches with all precipitation treatments represented in each batch. Planting was completed on June 25 and all plants were watered one last time. On July 12, plants were fertilized with 2.5 mL Scott's Azalea, Camellia, and Rhododendron food (16−2−3 N−P−K). In late August, there was a long dry period (Fig. S2), so to avoid mortality of tea plants

we watered each of the 100%, 75%, and 50% drought treatment plants with 200, 150, and 100 mL water, respectively, on August 22 and 25.

2.3 Growth and physiology data

Height from soil surface, and number of leaves were measured at the start and near the conclusion of the experiment (July 2 and August 17, respectively). Chlorophyll content of mature leaves was measured using a SPAD-502 chlorophyll meter (Spectrum Technologies, INC., Plainfield, IL) on August 25. Net assimilation rate and stomatal conductance were measured with a Li-6400xt infrared gas analyzer (LI-COR, Lincoln, Nebraska) on August 25 on a random subset of three plants per plot (N = 9 per treatment). Physiological data (net assimilation rate, stomatal conductance, chlorophyll content) were taken on three mature leaves with three replicate measures per leaf averaged before analysis.

2.4 MeJA treatment

Methyl jasmonate (MeJA) has been used in many studies as a simulated herbivory treatment in order to elicit plant defense responses, including studies on tea (Ahmed et al., 2014a; Cai et al., 2013; Kfoury et al., 2017). On August 30, a random sample of six potted tea plants per plot were randomly assigned either a control or MeJA treatment and removed from their plots to be grouped by treatment. MeJA treatment plants were sprayed until runoff with a solution of 200 μM MeJA in 10% ethanol and 0.125% Triton-X (Sigma-Aldrich, St. Louis, MO), and control plants were sprayed until runoff with a control solution of 10% ethanol and 0.125% Triton-X (plants had about 50 leaves). Ethanol and Triton-X were included as surfactants to improve penetration of MeJA through the leaf cuticle. At the time of spray application, control plants

were about 50 m upwind of MeJA plants to avoid induction of control plants by volatilized MeJA.

2.5 Volatile Collection

Roughly 24 hours after MeJA treatment, at 10:30 am, volatiles were sampled from the underside of the second expanded leaf from the apical meristem of tea plants by direct contact sorptive extraction (DCSE) using 0.5 mm thick, 10 mm long, polydimethylsiloxane coated stir bars (Twisters, Gerstel, Mülheim an der Ruhr, Germany) (Kfoury et al., 2017). Twisters were held on leaves with two small (2 mm dia.) neodymium magnets placed on the upper leaf surface. A field blank was collected by suspending a Twister in the air from a string with a magnet at the same height of the other Twisters. Twisters were recovered 2 hours later and sealed in vials for transport to the lab. Weather during sampling was partly cloudy with a mean air temperature of 18ºC. Before DCSE, one plant was excluded because it dropped a large number of leaves after MeJA application. During sample collection, five Twisters were lost or contaminated and were not analyzed. Prior to gas chromatography/mass spectrometry (GC/MS) analysis, each Twister was directly spiked with 1 µL of 10 µg/ml naphthalene-d_8 (Restek, Bellefonte, PA), which was used as an internal standard.

2.6 Gas chromatography/mass spectrometry

Twisters were thermally desorbed onto a GC/MS using operating parameters identical to those used in Kfoury et al. (2017). Peaks were identified by spectral deconvolution using Ion Analytics (Gerstel) software, as described previously (Kowalsick et al., 2014), using a database of 605 compounds found in tea plant samples in previous comprehensive analyses of tea plant volatile

metabolite chemistry (Kfoury et al., 2018b). Target compounds found in samples were then subtracted from the total ion current signal and the remaining signal was used for untargeted analysis (Kfoury et al., 2018a; Robbat et al., 2017). The retention index (RI) of each compound was calculated using a standard mix of C_7-C_{30} *n*-alkanes (Sigma-Aldrich).

2.7 Non-volatile sample collection and extraction

Young leaves (1^{st}–4^{th} leaves from the apical meristem on all stems, about 15–30 leaves) were harvested by gloved hand and put into a cooler with dry ice before being transported back to the lab. Leaves were stored at -80ºC then lyophilized for 48 hours and pulverized in a ball grinder (KLECO Visalia, CA) for 30 seconds and stored in well-sealed amber glass vials at -5ºC. Each sample was extracted in triplicate using the method described in Kfoury et al. (2018b), with an extraction solvent of 80% methanol/water (v/v) with 0.1% formic acid (Sigma-Aldrich). A 1/10 dilution of the extract was prepared for injection into the liquid chromatograph/mass spectrometer (LC/MS) using the same 80% methanol, 0.1% formic acid solution. Samples were spiked with 20 µg/ml paraxanthine (Sigma-Aldrich, ~98%), which was used as an internal standard.

2.8 Liquid chromatography/mass spectrometry

Non-volatile separations and quantitation were performed on an Agilent Technologies (Santa Clara, CA) 1260 Infinity II HPLC, consisting of a binary pump, a chilled autosampler, a temperature-controlled column compartment, and a diode array detector (DAD), coupled with an Agilent Technologies 6120 quadrupole MS and electrospray ionization (ESI) source. 1 µL of sample, stored at 4 °C in an autosampler tray, was injected onto a superficially porous C18

column (150 mm × 3.0 mm i.d., 2.7 μm d_p, Agilent Technologies), with temperature set to 32 °C. The mobile phase consisted of 0.1% formic acid in water (v/v) (A), and methanol (B), pumped at a flow rate of 0.5 mL/min, with a gradient elution program as follows: 6-35.7% B (0-22 min), 35.7-100% B (22-23 min), 100% B (23-28 min), with a re-equilibration time of 7 min.

ESI parameters were optimized by a flow-injection analysis to maximize ionization of target compounds. The drying gas temperature was set to 350 °C, with a flow rate of 12.0 L/min, and nebulizer pressure of 55 psi. The capillary voltage was set to 3 kV. Mass spectra were acquired in positive ionization mode from 100 to 1000 *m/z* at a rate of 0.943 spectra/sec, with a fragmentation voltage of 120 V.

Caffeine (Alfa-Aesar, Ward Hill, MA, 99%), theophylline (Sigma-Aldrich, ≥ 99%), theobromine (Sigma-Aldrich, ≥ 99.0%), L-theanine (Sigma-Aldrich, ≥ 98%), gallic acid (Fluka Analytical, Morris Town, NJ, 97.9% ± 0.6%), gallocatechin (Indofine, Hillsborough Township, NJ >99%), catechin (ChromaDex, Irvine, CA, 94.9%), catechin gallate (Indofine, >98%), gallocatechin gallate (ChromaDex, 98.4%), epigallocatechin (ChromaDex, 94.6%), epicatechin (ChromaDex, 96.2%), epigallocatechin gallate (Sigma-Aldrich, ≥95%), and epicatechin gallate (ChromaDex, 96.0%) were quantified with reference to quadratic calibration curves constructed from the analysis of standard mixtures serially diluted to yield seven calibrant levels (at least five included in the calibration). These compounds were chosen because of their importance to both ecological interactions as well as tea quality and effects on human health (Scott and Orians, 2018). Calibration samples were spiked with 20 μg/ml of paraxanthine internal standard (a compound not found in tea) and injected under conditions identical to the tea samples. Correlation

coefficients for calibration curves ranged from 0.996 to 1.000. Target compounds were quantified based on the *m/z* $[M+H]^+$ ion abundance, with compound identities confirmed using two qualifier fragment ions present at abundance ratios consistent with those observed in the standards, utilizing three ions total for detection. MassHunter (Agilent Technologies) software was used to analyze data files.

2.9 Data pre-treatment

For both LC/MS and GC/MS data, only compounds detected in more than three samples were included in the datasets.

For GC/MS data, relative peak area (RPA) was calculated by dividing peak areas by the area of the internal standard, naphthalene-d_8. RPAs of compounds in the field blank were subtracted from samples and any resulting values less than or equal to 0 were considered non-detects. Non-detects were replaced with a small value equal to 1 divided by the area of the internal standard in order to allow for log-transformation and multivariate analysis. The data were then auto-scaled. Three outliers were identified from a preliminary PCA using the outlier plot provided by the *ropls* R package (Thévenot et al., 2015), confirmed with univariate scatterplots (not shown), and removed, yielding a final dataset of 45 samples and 128 metabolites.

For LC/MS data, one replicate extraction was excluded due to a dilution error. Triplicate extractions were summarized by calculating a mean for each biological replicate (individual tea plant) and auto-scaled before analysis. Two outliers were identified from a preliminary PCA using the outlier plot provided by the *ropls* package, confirmed with univariate scatterplots (not

shown), and removed, yielding a final dataset of 51 samples and 10 metabolites. Concentrations of metabolites lower than the lowest calibration standard are estimated by extrapolation of the calibration function.

2.10 Statistical analysis

All statistical analyses were conducted in R (R Core Team, 2018). Linear mixed effects models were conducted using the *lme4* package (Bates et al., 2017). A model selection approach was used for analysis of physiological data (chlorophyll content, net assimilation rate, and stomatal conductance) starting with drought treatment as a fixed effect and plot and plant ID as random effects. We used Akaike's information criterion (AIC) to select the appropriate random effects, followed by the significance of the precipitation treatment compared to an intercept-only model. Stomatal conductance was log transformed before analysis to improve normality and heteroscedasticity. Significance tests of best-fit models were conducted using the *Anova* function in the *car* package (Fox and Weisberg, 2017).

Plant growth was assessed by measuring change in height and change in leaf count from July 2 to August 17. For both of these response variables, a model selection approach was taken starting with drought treatment as a fixed effect and plot ID as a random effect. Significance of best-fit models was determined by analysis of variance (ANOVA) for linear models and Analysis of Deviance for linear mixed effects models. Pairwise post-hoc tests were conducted with the *emmeans* package (Lenth, 2018).

Principal component analysis (PCA) was conducted using the *ropls* package (Thévenot et al., 2015) and PERMANOVA was conducted using the *adonis* function from the *vegan* package using 999 permutations after testing for the assumption of homogeneity of dispersion using the *betadisper* function (Oksanen et al., 2018). Post-hoc PERMANOVAs were conducted to explore interactive effects of drought and MeJA treatment using the *pairwiseAdonis* package, and false discovery rate adjusted p-values are reported (Martinez Arbizu, 2017).

Partial least squares discriminant analysis (PLS-DA) was conducted using the *ropls* package in order to confirm a main effect of MeJA treatment on tea metabolites. Variable importance in projection (VIP) scores were extracted from the PLS-DA model and a cutoff of VIP > 1 was used to identify important metabolites involved in response to MeJA treatment (Chong and Jun, 2005).

3. Results

3.1 Physiological effects of drought stress

During the experimental period between June 18 and August 25, the BACE facility received a total of 157.2 mm of rain with a mean daily rainfall of 2.42 ± 7.22 mm/day (Fig S2). Therefore, the 75% and 50% ambient rainfall plots received a mean of 1.81 and 1.20 mm/day, respectively. The mean daily average air temperature for the duration of the experiment was 22.78 ± 2.61ºC (Fig S2).

The models that best explained the effects of drought on chlorophyll content (SPAD units) and net assimilation rate (photosynthetic activity) included precipitation treatment as a fixed effect and plant ID as a random effect. Net assimilation rate was reduced significantly by reduced

precipitation (Analysis of deviance, $X^2 = 20.65$, df = 2, $p < 0.0001$) (Fig 1A). The best-fit model for log-transformed stomatal conductance included plot and plant ID as random effects and precipitation treatment as a fixed effect. Log-transformed stomatal conductance was significantly reduced by decreased precipitation (Analysis of deviance, $X^2 = 6.66$, df = 2, $p = 0.036$) (Fig 1B). There was no significant effect of precipitation treatment on chlorophyll content (Analysis of deviance, $X^2 = 0.28$, df = 2, $p = 0.870$) (Fig 1C).

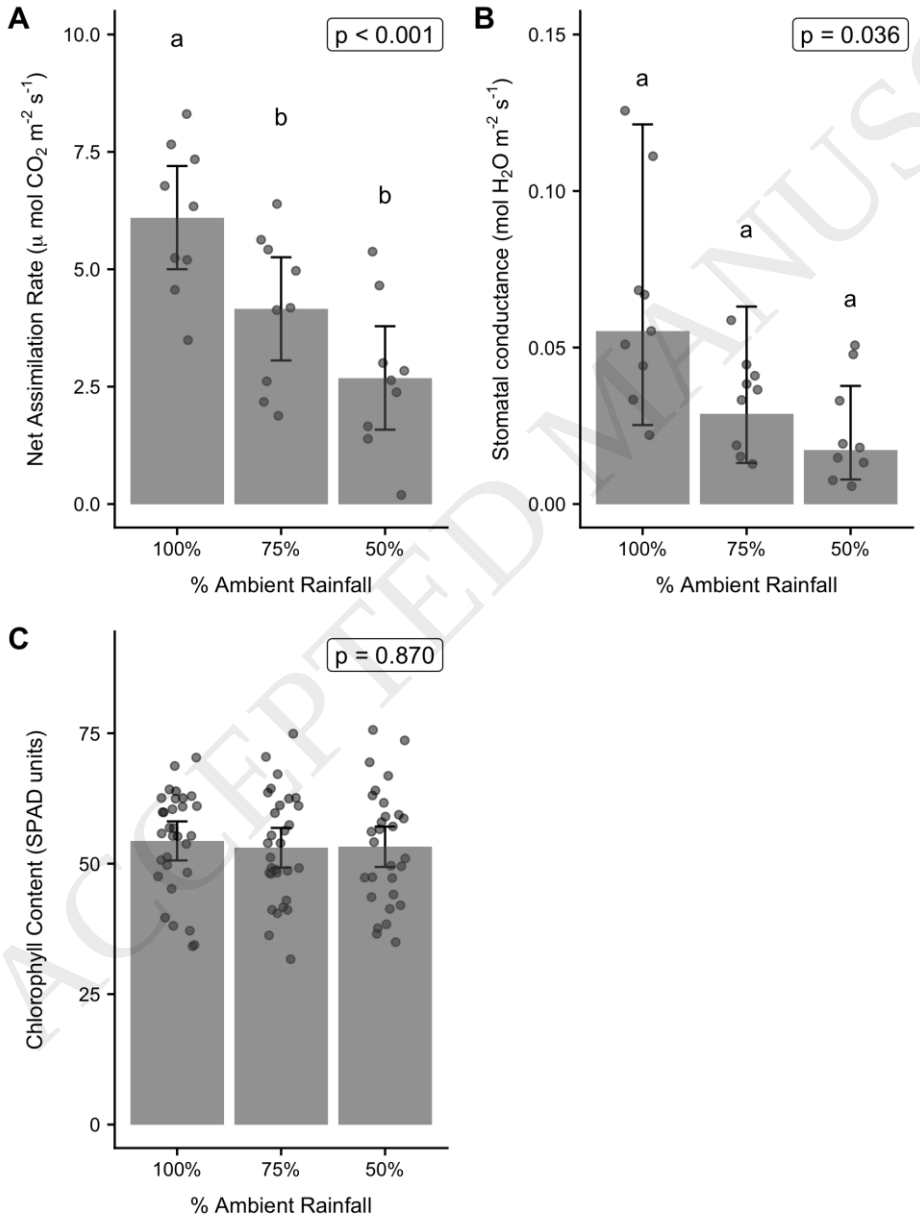

Figure 1: Effects of precipitation treatment on tea plant physiology. Plant level means (points), treatment means (bar heights) and 95% confidence intervals for net assimilation rate (A), stomatal conductance (B), and chlorophyll content (C) of tea plants grown at different precipitation regimes. For A and B, n= 9 plants per treatment. For C, n = 90 plants per treatment. Confidence intervals and p-values are results from Analysis of Deviance. Different letters indicate significant differences between groups (Tukey tests, $p < 0.05$). Analysis of Deviance for stomatal conductance (B) was conducted on log transformed data and confidence intervals are back-calculated to the untransformed scale.

3.2 Growth

The model that best explained change in height included only the main effect of precipitation treatment. For change in leaf count, the best-fit model included precipitation as a fixed effect and plot ID as a random effect. Precipitation treatment had a significant effect on growth (ANOVA, $F = 3.77$, $df = 2$, $p = 0.027$) and change in leaf count (Analysis of Deviance, $X^2 = 10.21$, $p = 0.007$). Plants in the 100%, 75%, and 50% rainfall treatments had a mean change in height of 5.83 cm, 3.53 cm, and 3.13 cm, respectively (Fig. 2A) and increased their leaf count by an average of 25.5, 18.0, and 11.2 leaves, respectively (Fig. 2B).

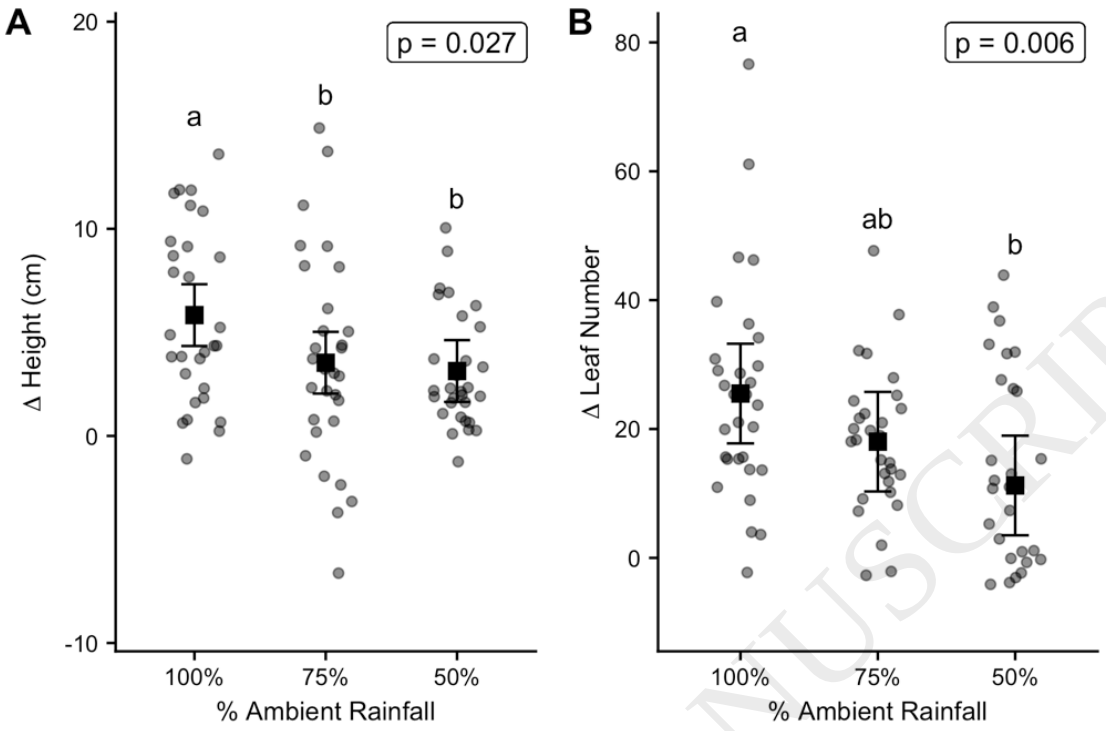

Figure 2. Effects of precipitation treatment on tea plant growth. Means (boxes) and 95% confidence intervals are shown for change in height (A) and change in leaf count (B) between July 2 and Aug 17 of tea plants grown under three precipitation regimes. The p-values are results from Analysis of Deviance tests. Different letters indicate significant differences between groups (Tukey tests, $p < 0.05$).

3.3 Volatile metabolites

A total of 128 volatile metabolites were found, with 99 identified (61 of which are confirmed with reference standards) and 29 unknowns (Table S1). A total of 46 volatile metabolites were common among all samples. In a plot of the first two PCA axes, the best separation of treatments was along PC1, which explained 16% of total variation in metabolite profile (Fig. 3). MeJA treatment affected volatile metabolite profiles only for the 100% rainfall treated plants along PC1. No obvious separation by herbivory treatment was observed for the 75% and 50% rainfall

treatments and no separation by precipitation treatment was obvious. This pattern was confirmed by 2-way PERMANOVA showing a significant main effect of MeJA treatment ($F = 3.26$, $df = 1$, $p = 0.001$), and a marginally significant interaction between MeJA and precipitation treatments ($F = 1.32$, $df = 2$, $p = 0.058$) but no significant main effect of precipitation on volatile metabolites ($F = 1.00$, $df = 2$, $p = 0.435$). Post-hoc pairwise PERMANOVAs were conducted to test for an effect of MeJA treatment within each level of precipitation treatment. This confirmed significant separation at 100% ambient rainfall ($F = 3.26$, $df = 14$, $p = 0.003$), but no significant separation at 75% ambient rainfall ($F = 1.45$, $df = 15$, $p = 0.108$) or at 50% ambient rainfall ($F = 1.32$, $df = 13$, $p = 0.128$).

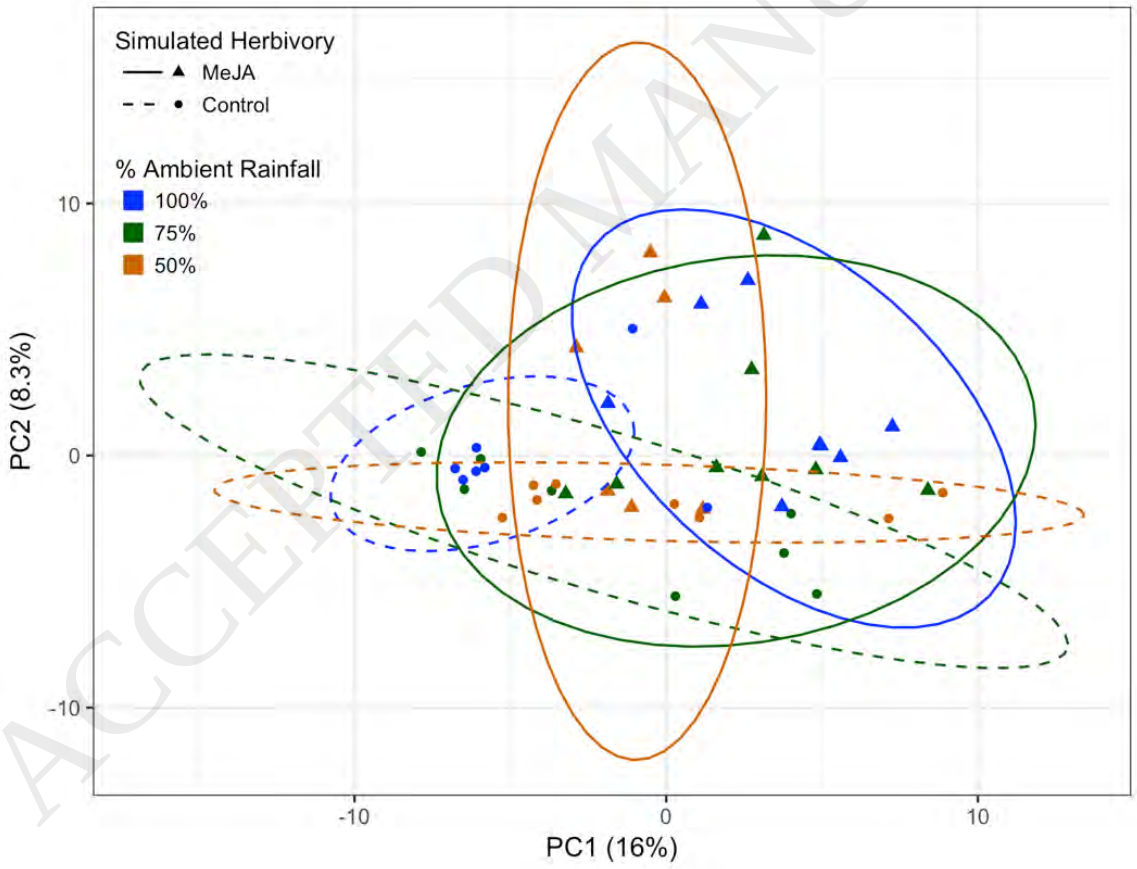

Figure 3. Score plot of principal component analysis based on relative peak areas of 128 volatile metabolites (log-transformed, scaled RPA) for tea plants grown under three precipitation regimes

(% ambient rainfall: 100% in blue, 75% in green, 50% in orange) and treated with either methyl jasmonate (triangles, solid ellipses) or a control spray (circles, dashed ellipses). Ellipses represent 95% confidence based on a multivariate t-distribution.

The PLS-DA model showed significant separation between control and MeJA sprayed plant volatile blends ($R^2 = 0.84$, $Q^2 = 0.65$, $p_{Q2} = 0.001$) and resulted in 42 metabolites with VIP scores greater than 1 (Table 1). The induced response to herbivory of many compounds appears to have been inhibited by severe drought stress. For example, MeJA induction of dodecanal, benzaldehyde, and anthracene is much less in the 50% rainfall treatment compared to the other two rainfall treatments. This pattern whereby a compound is induced by MeJA under moderate drought and either not induced or reduced in concentration by MeJA under severe drought occurs for 21 of the 42 compounds with VIP scores greater than 1. The induced response to MeJA is stronger in severely drought-stressed plants for only six compounds (mesityl oxide, (3-hydroxy-2,4,4-trimethylpentyl) 2-methylpropanoate, cis-methyl dihydrojasmonate, (1-hydroxy-2,4,4-trimethylpentan-3-yl) 2-methylpropanoate, methyl salicylate, and 2-ethylhexanol). We also see some metabolites that appear to have a response to herbivory somewhat independent of drought conditions. For example, ethyl-4-ethoxybenzoate and mesityl oxide are induced by MeJA regardless of precipitation treatment. Other compounds are reduced in concentration by MeJA treatment compared to control like trans-α-bergamotene, dihydroisophorone, 9 (an unidentified compound), cyclopentanone, α-dimethylbenzyl alcohol, and limonene. Some compounds appear to be induced by both drought and MeJA such as furfural, and 1-octadecanol.

3.4 Non-Volatile metabolites

Caffeine, catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallocatechin, and L-theanine were detected in all samples (Table 2). Theobromine was only detected in 38 of the 51 samples and gallic acid was only detected in 6 samples. No samples contained detectible levels of catechin gallate, gallocatechin gallate, or theophylline and those compounds were therefore removed from the dataset. Catechin, gallic acid, and theobromine concentrations were lower than the lowest calibration standard in all samples, therefore, these metabolites were removed from the dataset as well. Although L-theanine concentrations were low enough to be determined by extrapolation in eight of the 43 samples it was detected in, it was included in the dataset.

In PCA of non-volatiles, the first two PC axes explain 32% and 26% of variation, respectively. There is no separation by precipitation treatment or MeJA treatment visible in the PCA (Fig 4). This is confirmed by two-way PERMANOVA, which shows no significant effect of precipitation treatment ($F = 1.37$, $df = 2$, $p = 0.194$), MeJA treatment ($F = 0.31$, $df = 1$, $p = 0.918$), or their interaction ($F = 0.58$, $df = 2$, $p=0.844$).

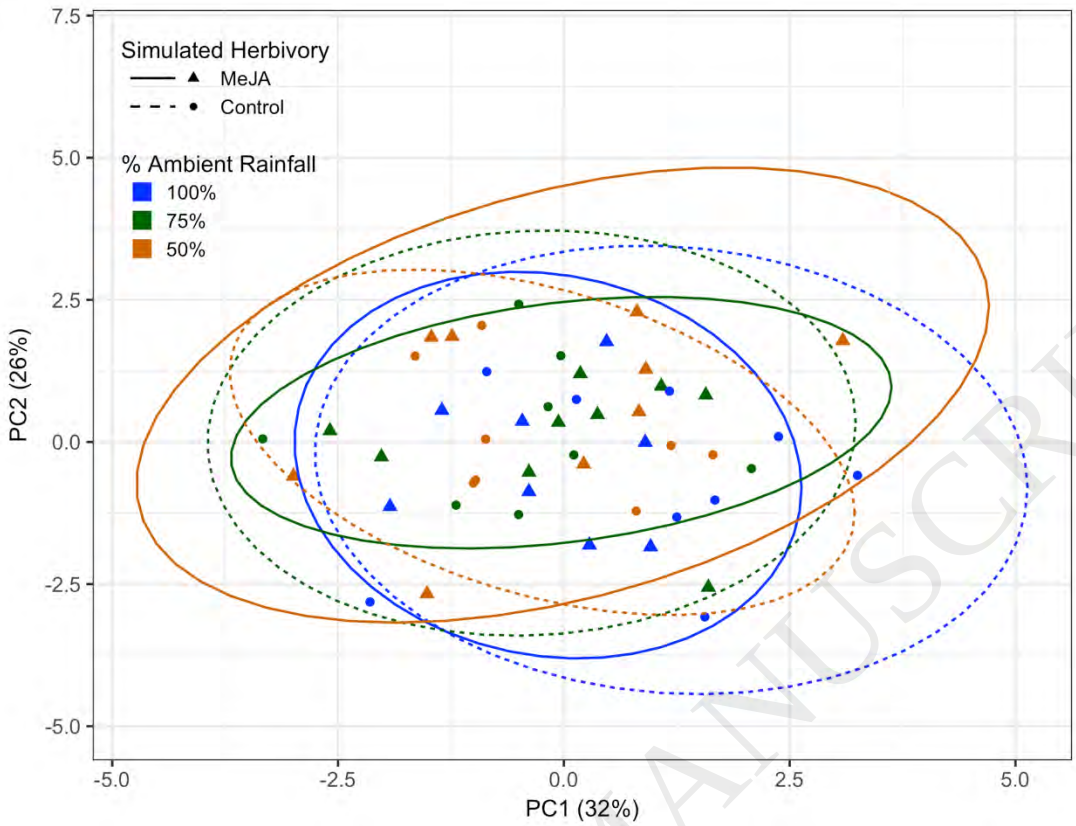

Figure 4. Score plot of a principal component analysis based on concentrations of seven non-volatile metabolites in tea plants grown under three precipitation regimes (% ambient rainfall: 100% in blue, 75% in green, 50% in orange) and treated with either methyl jasmonate (triangles, dashed ellipses) or a control spray (circles, solid ellipses). Ellipses represent 95% confidence based on a multivariate t-distribution.

4. Discussion

In this study we found interactive effects of drought stress and MeJA on volatile metabolites and no effect of drought or MeJA on select non-volatile metabolites (catechins, methylxanthines, and L-theanine). Severely drought-stressed plants showed no significant response to MeJA treatment while the less stressed plants showed many MeJA induced changes in volatile profile.

4.1 Effect of drought on growth, physiology and baseline metabolites

Precipitation treatments successfully created physiological drought stress in tea plants. Ambient rainfall was low (mean = 2.42 mm/day) resulting in a mean stomatal conductance indicative of moderate drought stress (Griesser et al., 2015). Plants in the 75% and 50% rainfall treatments had a mean stomatal conductance indicative of severe drought stress. While we observed treatment differences in growth, net assimilation rate, and stomatal conductance, we did not observe a difference in chlorophyll content, which is potentially an indicator of drought tolerance (Arunyanark et al., 2008). Similarly, there was no effect of precipitation treatment alone on volatile or non-volatile metabolite profiles. These results are unusual but not unexpected.

The effects of drought on volatile emissions in the literature range from decreases in volatile emissions with drought stress (Yani et al., 1993), to no effect of drought (Marchese et al., 2010), to an induction of volatiles by drought stress (Cao et al., 2007; Copolovici et al., 2014; Lavoir et al., 2009; Munné-Bosch and Peñuelas, 2003). Observed responses of non-volatiles to water stress also vary depending on plant genotype, degree of water stress, and metabolite identity. For example, Ahmed et al. (2014a) found significant reductions in total methylxanthines, and an increase in epicatechin gallate and total phenolics of tea plants under drought stress, but no significant impact on epigallocatechin gallate concentrations. To the contrary, Popović et al. (2016) found that drought stress reduced total polyphenol content of poplar plants with the exception of a few flavonoids that increased in concentration in the roots of one genotype. Chakraborty et al. (2002) observed an increase in total phenolics (determined by Folin-Ciocaleau method, a non-selective assay) under moderate drought and a drastic decrease under severe drought in tea plants.

In our study, even tea plants grown in the 100% rainfall treatment were moderately drought-stressed. It is therefore important not to extrapolate to unstressed plants. It is possible that the effects of drought stress on tea metabolites would have been more obvious had there been higher ambient rainfall.

4.2 Interactive effects of drought and simulated herbivory on induced metabolites

What is most striking about our results is the interactive effects of drought and MeJA treatment on volatile metabolites. We found that MeJA only affected volatile emissions for plants under moderate drought stress (100% rainfall). About half of the metabolites important for separating MeJA from control treated plants (VIP > 1) increased in concentration due to MeJA in the 100% and 75% rainfall treatments but were either reduced or unaffected in the 50% rainfall treatment. MeJA is well known to induce changes in tea plant volatile release (Cai et al., 2013; Kfoury et al., 2017) so the lack of an effect of MeJA in the 75% and 50% precipitation treatments shows that severe drought stress can suppress volatile induction.

Only six of the important compounds showed increased induction by MeJA in the 50% rainfall treatment compared to the 100% rainfall treatment including methyl salicylate, an important plant hormone involved in anti-herbivore defense (Park et al., 2007). Copolovici et al. (2014) found that herbivory induced a greater production of volatiles, including methyl salicylate, in drought-stressed *Alnus glutinosa* trees compared to well-watered individuals. Mewis et al. (2012) also found that methyl salicylate was induced by aphid feeding on *Arabidopsis thaliana* only under drought conditions. We found a similar pattern in our study, whereby the mean relative

peak area of methyl salicylate in MeJA treated, 50% ambient rainfall tea plants was an order of magnitude higher than any other treatment. This was largely driven by a single sample but may still be indicative of priming by drought stress. Production of methyl salicylate by plants leads to a systemic acquired resistance that helps defend against future attack by pathogens or piercing-sucking herbivores like aphids (Park et al., 2007). Drought-stressed plants may be especially susceptible to attack by aphids due to increased osmolyte concentrations in phloem sap, and this could explain an increase in inducibility of methyl salicylate under drought stress (Mewis et al., 2012). Our data show that for a small subset of metabolites, priming by drought stress increased inducibility, possibly due to hormonal cross-talk.

Although about half of the 42 volatile metabolites important for separating control from MeJA sprayed plants showed one of these two patterns of induction (i.e., less inducible under severe drought stress or more inducible under severe drought stress), there was no consistent pattern among the other volatile metabolites. Some are only produced by MeJA treated plants, some only by control treated plants; some increase or decrease monotonically as drought stress increases; some are found at their highest concentration in the intermediate rainfall treatment. Essentially, when looking at the induction profiles of individual volatiles across our drought stress gradient, every possible pattern of induction is observed. This inconsistent effect of stress on tea volatile metabolites is similar to what has been observed in previous work on tea volatiles (Kowalsick et al., 2014). Using a targeted/untargeted approach for metabolite analysis allows one to avoid drawing conclusions based on the idiosyncratic responses of a small subset of plant metabolites.

In contrast, the select non-volatile metabolites we measured were not induced by MeJA treatment in any of the precipitation treatments. Compared to volatiles, phenolics and methylxanthines in tea may be less inducible by MeJA. Previous research on glasshouse-grown potted tea plants also showed little effect of MeJA on phenolics and methylxanthines (Ahmed et al., 2014a). The authors of that study concluded that the lack of response may have been an artifact of growing plants under low UV conditions. However, our experimental design exposed plants to UV radiation and we still did not detect an effect of MeJA. Induction of tea catechins by herbivory has been commonly observed in experiments using a variety of insect herbivores (Reviewed in Scott and Orians, 2018) and it is possible that mechanical damage or elicitors may be a necessary component of non-volatile induction in tea in addition to activation of the JA pathway. It is also possible that drought stress limits the ability of tea plants to respond to MeJA with induction of non-volatile metabolites, similar to what we observed with the volatile metabolites, but that we were unable to capture this pattern with our experiment since all the plants in our study experienced some degree of drought stress. Future studies exposing plants to a gradient of drought stress crossed with herbivory could further elucidate the interactive effects of drought and herbivory on non-volatile metabolite induction. Caution should be exercised when drawing conclusions based on only one level of drought stress.

Changes in the tea secondary metabolites we measured can be extremely important in determining crop quality and farmer income (Ahmed et al., 2014b; Naldi et al., 2014). Although tea experiences significant reductions in yield due to drought and insect herbivory (Duncan et al., 2016; Hazarika et al., 2009), these environmental stresses may improve crop quality through induction of secondary metabolites (Bertin et al., 2000; Savoi et al., 2016; Scott and Orians, 2018). Therefore, a better understanding of the interactive effects of environmental stresses on

plant metabolites could help determine optimal levels of stress that balance quality and yield to improve farmer income and produce better quality crop products.

5. Conclusion

We found complex, interactive patterns of volatile metabolite induction by simulated herbivory in plants under different degrees of drought stress. Plants experiencing severe drought had no significant induced response to MeJA while plants experiencing only moderate drought showed an induced change in volatile profile. This supports our prediction that severe drought stress results in a reduced ability to respond to herbivory by inducing the production of secondary metabolites, possibly due to carbon limitation. We note, however, that severe drought stress increased the induction of a small subset of compounds including methyl salicylate, mesityl oxide, (3-hydroxy-2,4,4-trimethylpentyl) 2-methylpropanoate, cis-methyl dihydrojasmonate, (1-hydroxy-2,4,4-trimethylpentan-3-yl) 2-methylpropanoate, and 2-ethylhexanol. Therefore, the increased inducibility under drought stress of even a small subset of compounds could have important implications for plant defense against aphids. It was surprising that the non-volatile metabolites L-theanine, catechins, and methylxanthines were unaffected by both drought and MeJA treatment, which differs from most results in the literature. It is possible that the effect of drought on these non-volatiles is non-linear—i.e., distinct metabolite profiles in unstressed plants compared to plants experiencing any degree of drought stress—and we did not capture this response in our study.

CRediT Statement

Eric R Scott: Conceptualization, methodology, software, investigation, formal analysis, data curation, writing–original draft preparation, visualization. **Li Xin:** Conceptualization, methodology, investigation, writing–review and editing. **Nicole Kfoury:** Methodology, investigation, writing–review and editing. **Joshua Morimoto:** Methodology, investigation, writing–review and editing. **Wen-Yan Han:** Conceptualization, supervision. **Selena Ahmed:** supervision, funding acquisition. **Sean B Cash:** Supervision, funding acquisition, writing–review and editing. **Timothy S. Griffin:** Supervision, funding acquisition. **John R. Stepp**: Supervision, funding acquisition, writing–review and editing. **Albert Robbat Jr.:** Supervision, funding acquisition, resources, writing–review and editing, software, methodology**. Colin M. Orians:** Conceptualization, methodology, investigation, writing–review and editing, supervision, funding acquisition, resources.

6. Acknowledgments

This work was supported by the National Science Foundation, United States Grant BCS-1313775. We thank Jeffrey S. Dukes for allowing us to conduct this research as part of the Boston-Area Climate Experiment, which was supported by grants from the National Science Foundation (DEB-0546670); the U.S. Department of Energy's Office of Science (BER), through the Northeastern Regional Center of the National Institute for Climatic Change Research and the Terrestrial Ecosystem Sciences program; and the United States Department of Agriculture's National Institute of Food and Agriculture (USDA-NIFA 2015-67003-23485). We also thank the University of Massachusetts' Extension Division for leasing land to the BACE. Michael LaScaleia helped with planting and data collection. Michelle Mu and Annie

Nguyen assisted with sample collection and data collection in the field. Risa McNellis assisted with LI-6400xt operation.

7. Author contributions

E.R. Scott, X. Li, and C. M. Orians conceived, designed, and carried out the field experiment. N. Kfoury and J. Morimoto performed chemical analysis of volatile and non-volatile metabolites. E.R. Scott analyzed the data and led the writing of the manuscript with contributions from C.M. Orians, N. Kfoury, J. Morimoto, and A. Robbat Jr. All authors contributed significantly to the drafts and gave their final approval for publication.

8. Competing interests

The authors have no competing interests to declare.

9. Works Cited

Ahmed, S., Orians, C.M., Griffin, T.S., Buckley, S., Unachukwu, U., Stratton, A.E., Stepp, J.R., Robbat, A., Cash, S., Kennelly, E.J., 2014a. Effects of water availability and pest pressures on tea (*Camellia sinensis*) growth and functional quality. AoB Plants 6, plt054-plt054. https://doi.org/10.1093/aobpla/plt054

Ahmed, S., Stepp, J.R., Orians, C.M., Griffin, T., Matyas, C., Robbat, A., Cash, S.B., Xue, D., Long, C., Unachukwu, U.J., Buckley, S., Small, D., Kennelly, E., Robbat Jr, A., Cash, S.B., Xue, D., Long, C., Unachukwu, U.J., Buckley, S., Small, D., Kennelly, E., 2014b. Effects of Extreme Climate Events on Tea (\emph{Camellia sinensis}) Functional Quality Validate Indigenous Farmer Knowledge and Sensory Preferences in Tropical China. PLoS One 9, e109126. https://doi.org/10.1371/journal.pone.0109126

Arunyanark, A., Jogloy, S., Akkasaeng, C., Vorasoot, N., Kesmala, T., Nageswara Rao, R.C., Wright, G.C., Patanothai, A., 2008. Chlorophyll Stability is an Indicator of Drought Tolerance in Peanut. J. Agron. Crop Sci. 194, 113–125. https://doi.org/10.1111/j.1439-037X.2008.00299.x

Auyeung, D.S.N., Suseela, V., Dukes, J.S., 2013. Warming and drought reduce temperature sensitivity of nitrogen transformations. Glob. Chang. Biol. 19, 662–676. https://doi.org/10.1111/gcb.12063

Bates, D., Maechler, M., Bolker, B., Walker, S., 2017. lme4: Linear Mixed-Effects Models using "Eigen" and S4.

Bennett, R.N., Wallsgrove, R.M., 1994. Tansley Review No. 72. Secondary metabolites in plant defence mechanisms. New Phytol. 127, 617–633. https://doi.org/10.2307/2558194

Bertin, N., Guichard, S., Leonardi, C., Longuenesse, J.J., Langlois, D., Navez, B., 2000. Seasonal evolution of the quality of fresh glasshouse tomatoes under Mediterranean conditions, as affected by air vapour pressure deficit and plant fruit load. Ann. Bot. 85, 741–750. https://doi.org/10.1006/anbo.2000.1123

Cai, X.-M., Sun, X.-L.L., Dong, W.-X.X., Wang, G.-C.C., Chen, Z.-M.M., 2013. Herbivore species, infestation time, and herbivore density affect induced volatiles in tea plants. Chemoecology 24, 1–14. https://doi.org/10.1007/s00049-013-0141-2

Camfield, D.A., Stough, C., Farrimond, J., Scholey, A.B., 2014. Acute effects of tea constituents L-theanine, caffeine, and epigallocatechin gallate on cognitive function and mood: A systematic review and meta-analysis. Nutr. Rev. 72, 507–522. https://doi.org/10.1111/nure.12120

Cao, P., Liu, C., Liu, K., 2007. Aromatic constituents in fresh leaves of Lingtou Dancong tea

induced by drought stress. Front. Agric. China 1, 81–84. https://doi.org/10.1007/s11703-007-0015-x

Chakraborty, U., Dutta, S., Chakraborty, B.N., 2002. Response of tea plants to water stress. Biol. Plant. 45, 557–562. https://doi.org/10.1023/A:1022377126056

Chong, I.-G., Jun, C.-H., 2005. Performance of some variable selection methods when multicollinearity is present. Chemom. Intell. Lab. Syst. 78, 103–112. https://doi.org/10.1016/J.CHEMOLAB.2004.12.011

Cipollini, D., 2010. Costs and benefits of induced resistance to herbivores and pathogens in plants. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 5, 1–25. https://doi.org/10.1079/PAVSNNR20105005

Copolovici, L., Kännaste, A., Remmel, T., Niinemets, Ü., 2014. Volatile organic compound emissions from *Alnus glutinosa* under interacting drought and herbivory stresses. Environ. Exp. Bot. 100, 55–63. https://doi.org/10.1016/j.envexpbot.2013.12.011

Dufresne, C.J., Farnworth, E.R., 2001. A review of latest research findings on the health promotion properties of tea. J. Nutr. Biochem. 12, 404–421. https://doi.org/10.1016/S0955-2863(01)00155-3

Duncan, J.M.A., Saikia, S.D., Gupta, N., Biggs, E.M., 2016. Observing climate impacts on tea yield in Assam, India. Appl. Geogr. 77, 64–71. https://doi.org/10.1016/j.apgeog.2016.10.004

Dunford, N.T., Vazquez, R.S., 2005. Effect of water stress on plant growth and thymol and carvacrol concentrations in Mexican oregano grown under controlled conditions. J. Appl. Hortic. 7, 20–22.

Fox, J., Weisberg, S., 2017. car: Companion to Applied Regression.

Griesser, M., Weingart, G., Schoedl-Hummel, K., Neumann, N., Becker, M., Varmuza, K., Liebner, F., Schuhmacher, R., Forneck, A., 2015. Severe drought stress is affecting selected primary metabolites, polyphenols, and volatile metabolites in grapevine leaves (*Vitis vinifera* cv. Pinot noir). Plant Physiol. Biochem. 88, 17–26. https://doi.org/10.1016/j.plaphy.2015.01.004

Han, W.-Y., Huang, J.-G., Li, X., Li, Z.-X., Ahammed, G.J., Yan, P., Stepp, J.R., 2017. Altitudinal effects on the quality of green tea in east China: a climate change perspective. Eur. Food Res. Technol. 243, 323–330. https://doi.org/10.1007/s00217-016-2746-5

Hazarika, L.K., Bhuyan, M., Hazarika, B.N., 2009. Insect Pests of Tea and Their Management 54, 267–284.

Hoeppner, S.S., Dukes, J.S., 2012. Interactive responses of old-field plant growth and composition to warming and precipitation. Glob. Chang. Biol. 18, 1754–1768. https://doi.org/10.1111/j.1365-2486.2011.02626.x

Karban, R., Agrawal, A.A., Mangel, M., 1997. The benefits of induced defenses against herbivores. Ecology 78, 1351–1355. https://doi.org/10.1890/0012-9658(1997)078[1351:TBOIDA]2.0.CO;2

Kfoury, N., Baydakov, E., Gankin, Y., Robbat, A., 2018a. Differentiation of key biomarkers in tea infusions using a target/nontarget gas chromatography/mass spectrometry workflow. Food Res. Int. 113, 414–423. https://doi.org/10.1016/j.foodres.2018.07.028

Kfoury, N., Morimoto, J., Kern, A., Scott, E.R., Orians, C.M., Ahmed, S., Griffin, T., Cash, S.B., Stepp, J.R., Xue, D., Long, C., Robbat, A., 2018b. Striking changes in tea metabolites due to elevational effects. Food Chem. 264, 334–341. https://doi.org/10.1016/j.foodchem.2018.05.040

Kfoury, N., Scott, E., Orians, C., Robbat, A., 2017. Direct Contact Sorptive Extraction: A Robust Method for Sampling Plant Volatiles in the Field. J. Agric. Food Chem. 65, 8501–8509. https://doi.org/10.1021/acs.jafc.7b02847

Kowalsick, A., Kfoury, N., Robbat, A., Ahmed, S., Orians, C., Griffin, T., Cash, S.B., Stepp, J.R., 2014. Metabolite profiling of Camellia sinensis by automated sequential, multidimensional gas chromatography/mass spectrometry reveals strong monsoon effects on tea constituents. J. Chromatogr. A 1370, 230–239. https://doi.org/10.1016/j.chroma.2014.10.058

Lavoir, A.-V., Staudt, M., Schnitzler, J.P., Landais, D., Massol, F., Rocheteau, A., Rodriguez, R., Zimmer, I., Rambal, S., 2009. Drought reduced monoterpene emissions from the evergreen Mediterranean oak Quercus ilex: results from a throughfall displacement experiment. Biogeosciences 6, 1167–1180. https://doi.org/10.5194/bg-6-1167-2009

Lenth, R., 2018. emmeans: Estimated Marginal Means, aka Least-Squares Means.

Lortzing, T., Steppuhn, A., 2016. Jasmonate signalling in plants shapes plant-insect interaction ecology. Curr. Opin. Insect Sci. 14, 32–39. https://doi.org/10.1016/j.cois.2016.01.002

Marchese, J.A., Ferreira, J.F.S., Rehder, V.L.G., Rodrigues, O., 2010. Water deficit effect on the accumulation of biomass and artemisinin in annual wormwood(Artemisia annua L., Asteraceae). Brazilian J. Plant Physiol. 22, 1–9. https://doi.org/10.1590/S1677-04202010000100001

Martinez Arbizu, P., 2017. pairwiseAdonis: Pairwise Multilevel Comparison using Adonis.

Mewis, I., Khan, M.A.M., Glawischnig, E., Schreiner, M., Ulrichs, C., 2012. Water Stress and Aphid Feeding Differentially Influence Metabolite Composition in *Arabidopsis thaliana* (L.). PLoS One 7. https://doi.org/10.1371/journal.pone.0048661

Munné-Bosch, S., Peñuelas, J., 2003. Photo- and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. Planta 217, 758–766. https://doi.org/10.1007/s00425-003-1037-0

Naldi, M., Fiori, J., Gotti, R., Périat, A., Veuthey, J.-L., Guillarme, D., Andrisano, V., 2014. UHPLC determination of catechins for the quality control of green tea. J. Pharm. Biomed. Anal. 88, 307–314. https://doi.org/10.1016/j.jpba.2013.08.054

Niinemets, Ü., 2015. Uncovering the hidden facets of drought stress: Secondary metabolites make the difference. Tree Physiol. 36, 129–132. https://doi.org/10.1093/treephys/tpv128

Nogués, I., Muzzini, V., Loreto, F., Bustamante, M.A., 2015. Drought and soil amendment effects on monoterpene emission in rosemary plants. Sci. Total Environ. 538, 768–778. https://doi.org/10.1016/j.scitotenv.2015.08.080

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2018. vegan: Community Ecology Package.

Park, S.-W.W., Kaimoyo, E., Kumar, D., Mosher, S., Klessig, D.F., 2007. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science (80-.). 318, 113–116. https://doi.org/10.1126/science.1147113

Pegoraro, E., Rey, A., Greenberg, J., Harley, P., Grace, J., Malhi, Y., Guenther, A., 2004. Effect of drought on isoprene emission rates from leaves of Quercus virginiana Mill. Atmos. Environ. 38, 6149–6156. https://doi.org/10.1016/j.atmosenv.2004.07.028

Popović, B.M., Štajner, D., Ždero-Pavlović, R., Tumbas-Šaponjac, V., Čanadanović-Brunet, J., Orlović, S., 2016. Water stress induces changes in polyphenol profile and antioxidant capacity in poplar plants (Populus spp.). Plant Physiol. Biochem. 105, 242–250.

https://doi.org/10.1016/j.plaphy.2016.04.036

R Core Team, 2018. R: A Language and Environment for Statistical Computing.

Reddy, A.R., Chaitanya, K.V., Vivekanandan, M., 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol. 161, 1189–1202. https://doi.org/10.1016/j.jplph.2004.01.013

Riemann, M., Dhakarey, R., Hazman, M., Miro, B., Kohli, A., Nick, P., 2015. Exploring Jasmonates in the Hormonal Network of Drought and Salinity Responses. Front. Plant Sci. 6, 1077. https://doi.org/10.3389/fpls.2015.01077

Robbat, A., Kfoury, N., Baydakov, E., Gankin, Y., 2017. Optimizing targeted/untargeted metabolomics by automating gas chromatography/mass spectrometry workflows. J. Chromatogr. A 1505, 96–105. https://doi.org/10.1016/j.chroma.2017.05.017

Savoi, S., Wong, D.C.J., Arapitsas, P., Miculan, M., Bucchetti, B., Peterlunger, E., Fait, A., Mattivi, F., Castellarin, S.D., 2016. Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (Vitis vinifera L.). BMC Plant Biol. 16, 67. https://doi.org/10.1186/s12870-016-0760-1

Scott, E., Orians, C., 2018. Differential Changes in Tea Quality as Influenced by Insect Herbivory, in: Han, W.-Y., Li, X., Ahammed, G.J. (Eds.), Stress Physiology of Tea in the Face of Climate Change (In Press). Springer Nature. https://doi.org/10.1007/978-981-13-2140-5

Strømme, C.B., Julkunen-Tiitto, R., Olsen, J.E., Nybakken, L., 2018. The dioecious Populus tremula displays interactive effects of temperature and ultraviolet-B along a natural gradient. Environ. Exp. Bot. 146, 13–26. https://doi.org/10.1016/J.ENVEXPBOT.2017.09.013

Thévenot, E.A., Roux, A., Xu, Y., Ezan, E., Junot, C., 2015. Analysis of the Human Adult Urinary Metabolome Variations with Age, Body Mass Index, and Gender by Implementing a Comprehensive Workflow for Univariate and OPLS Statistical Analyses. J. Proteome Res. 14, 3322–3335. https://doi.org/10.1021/acs.jproteome.5b00354

Weldegergis, B.T., Zhu, F., Poelman, E.H., Dicke, M., 2018. Correction to: Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. Oecologia 1–2. https://doi.org/10.1007/s00442-018-4149-8

Weldegergis, B.T., Zhu, F., Poelman, E.H., Dicke, M., 2015. Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. Oecologia 177, 701–713. https://doi.org/10.1007/s00442-014-3129-x

Yani, A., Pauly, G., Faye, M., Salin, F., Gleizes, M., 1993. The effect of a long- term water stress on the metabolism and emission of terpenes of the foliage of Cupressus sempervirens. Plant. Cell Environ. 16, 975–981. https://doi.org/10.1111/j.1365-3040.1993.tb00521.x

Zobayed, S.M.A., Afreen, F., Kozai, T., 2007. Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition. Environ. Exp. Bot. 59, 109–116. https://doi.org/10.1016/J.ENVEXPBOT.2005.10.002

Table 1. Relative peak area × 1000 ± standard error for compounds with a variable importance in projection (VIP) score > 1. VIP score is calculated based on a PLS-DA model that best explains separation between MeJA and control sprayed plants, regardless of precipitation treatment. A higher VIP score indicates a compound that better explains the separation between MeJA and control metabolite profiles. Experimental Van den Dool and Kratz retention indices (RI) are reported.

			100% Rainfall		75% Rainfall		50% Rainfall	
Compound	**RI**	**VIP**	**Control**	**MeJA**	**Control**	**MeJA**	**Control**	**MeJA**
Ethyl 4-ethoxybenzoate	1533	2.71	-	10.092 ± 1.19	-	5.346 ± 0.96	0.148 ± 0.03	3.785 ± 0.88
trans-α-Bergamotene	1448	2.34	0.285 ± 0.11	-	1.521 ± 0.17	-	1.384 ± 0.17	-
1-Dodecanol*	1480	1.93	0.185 ± 0.07	3.829 ± 0.36	0.795 ± 0.28	4.627 ± 0.79	2.812 ± 0.53	1.292 ± 0.21
Dodecanal*	1415	1.89	0.347 ± 0.07	1.774 ± 0.08	0.502 ± 0.12	1.812 ± 0.17	0.679 ± 0.14	0.605 ± 0.09
Dihydroisophorone	1042	1.87	4.878 ± 0.14	1.927 ± 0.35	5.637 ± 0.24	3.148 ± 0.27	6.38 ± 0.2	2.209 ± 0.37
1-Octadecanol	2084	1.66	-	1.636 ± 0.14	0.434 ± 0.09	1.374 ± 0.15	1.914 ± 0.57	0.825 ± 0.13
9	1032	1.66	15.527 ± 4.66	0.38 ± 0.11	3.162 ± 0.59	0.31 ± 0.08	4.707 ± 0.47	0.136 ± 0.04
Mesityl oxide*	803	1.63	-	6.669 ± 1.23	-	4.654 ± 1.08	-	10.736 ± 2.1
1-Hexadecanol*	1885	1.63	0.063 ± 0.02	2.404 ± 0.3	0.256 ± 0.04	2.419 ± 0.45	2.099 ± 0.74	2.167 ± 0.51
Furfural*	840	1.61	6.408 ± 1.85	20.586 ± 1.12	9.995 ± 1.24	17.738 ± 1.97	17.27 ± 2.19	15.097 ± 1.55
(3-hydroxy-2,4,4-trimethylpentyl) 2-methylpropanoate	1382	1.58	-	4.879 ± 1.14	-	3.505 ± 0.81	-	11.255 ± 2.74
Anthracene	1796	1.57	0.951 ± 0.31	4.818 ± 0.33	3.593 ± 0.48	5.558 ± 0.55	3.95 ± 0.49	2.175 ± 0.37
15	1148	1.56	-	3.038 ± 0.37	0.215 ± 0.08	2.297 ± 0.35	0.357 ± 0.1	-
2-Ethylhexyl-salicylate*	1817	1.56	-	1.19 ± 0.13	0.174 ± 0.04	0.813 ± 0.21	0.665 ± 0.17	0.221 ± 0.05
Homomenthyl salicylate*	1899	1.54	-	1.355 ± 0.19	0.112 ± 0.04	0.869 ± 0.27	0.731 ± 0.18	0.376 ± 0.09
Benzaldehyde*	965	1.53	0.091 ± 0.03	2.647 ± 0.25	0.065 ± 0.02	1.738 ± 0.21	1.647 ± 0.29	0.407 ± 0.11
Tridecanal	1516	1.52	0.128 ± 0.05	0.419 ± 0.04	0.045 ± 0.02	0.297 ± 0.05	0.155 ± 0.04	0.357 ± 0.07
Naphthalene	1189	1.50	-	1.285 ± 0.17	0.889 ± 0.17	1.79 ± 0.12	1.636 ± 0.27	0.653 ± 0.09

cis-Methyl dihydrojasmonate*	1660	1.49	-	0.866 ± 0.2	-	0.79 ± 0.14	-	1.171 ± 0.31
(1-hydroxy-2,4,4-trimethylpentan-3-yl) 2-methylpropanoate	1361	1.48	-	4.397 ± 1.02	-	4.314 ± 1	-	10.667 ± 2.53
2-Phenoxyethanol*	1229	1.47	-	3.027 ± 0.41	-	1.127 ± 0.26	0.901 ± 0.32	2.541 ± 0.6
11	1072	1.45	-	0.148 ± 0.03	1.382 ± 0.49	0.219 ± 0.02	0.154 ± 0.05	0.106 ± 0.02
3-Heptanone*	889	1.45	-	1.533 ± 0.14	1.223 ± 0.19	2.676 ± 0.34	0.964 ± 0.17	0.725 ± 0.12
α,α-Dimethylbenzyl alcohol*	1091	1.42	23.012 ± 1.29	14.255 ± 1.5	21.403 ± 1.3	18.243 ± 1.48	22.21 ± 0.51	9.416 ± 1.46
Cyclopentanone	797	1.39	1.509 ± 0.44	0.011 ± 0	0.53 ± 0.15	-	0.882 ± 0.15	0.056 ± 0.02
1,2-Cyclooctanedione	933	1.38	1.986 ± 0.75	10.671 ± 0.43	8.114 ± 0.93	13.69 ± 0.6	8.816 ± 1.2	6.259 ± 1.16
Limonene*	1032	1.38	15.571 ± 4.7	0.313 ± 0.08	3.152 ± 0.58	0.436 ± 0.07	4.391 ± 0.47	0.17 ± 0.04
2-Ethylhexyl benzoate	1720	1.29	0.04 ± 0.02	0.346 ± 0.04	0.147 ± 0.05	0.565 ± 0.1	0.192 ± 0.04	0.146 ± 0.04
18	1216	1.26	-	-	2.342 ± 0.83	-	0.489 ± 0.11	-
2(5H)-Furanone*	927	1.26	0.591 ± 0.16	3.344 ± 0.33	2.008 ± 0.29	1.952 ± 0.27	3.068 ± 0.71	0.553 ± 0.14
Methyl salicylate*	1201	1.26	-	3.675 ± 0.72	0.594 ± 0.21	2.157 ± 0.59	0.079 ± 0.03	38.894 ± 13.83
p-Cymene*	1029	1.25	20.569 ± 7.68	-	-	-	1.284 ± 0.36	-
p-Xylene*	871	1.22	-	2.304 ± 0.33	2.969 ± 0.56	2.714 ± 0.34	1.372 ± 0.44	0.789 ± 0.15
1	757	1.21	1.912 ± 0.72	16.746 ± 1.77	14.758 ± 2.28	14.508 ± 1.74	11.522 ± 2.67	2.609 ± 0.81
γ-Butyrolactone	923	1.21	1.497 ± 0.44	2.66 ± 0.25	1.72 ± 0.24	2.851 ± 0.26	3.396 ± 0.65	0.945 ± 0.21
13	1100	1.15	8.625 ± 2.73	18.379 ± 2.29	17.835 ± 2.43	11.711 ± 1.41	21.51 ± 4.56	10.4 ± 2.01
Styrene*	895	1.09	4.113 ± 0.86	2.804 ± 0.24	4.525 ± 0.51	3.495 ± 0.4	4.863 ± 0.67	1.024 ± 0.26
2-Ethylhexanol*	1032	1.03	1.881 ± 0.71	2.605 ± 0.59	0.273 ± 0.1	0.956 ± 0.32	0.505 ± 0.18	6.243 ± 1.65
Decanal*	1210	1.03	-	3.337 ± 0.59	2.554 ± 0.9	1.589 ± 0.56	1.084 ± 0.38	0.538 ± 0.22
Decanoic acid*	1372	1.03	0.489 ± 0.18	3.184 ± 0.39	1.209 ± 0.14	2.537 ± 0.27	2.389 ± 0.4	0.818 ± 0.2
26	1870	1.02	4.544 ± 0.51	13.614 ± 0.9	6.386 ± 0.66	10.58 ± 0.91	15.386 ± 1.59	6.957 ± 1.07
Capyrlic acid*	1178	1.00	0.255 ± 0.1	1.02 ± 0.13	0.052 ± 0.02	0.823 ± 0.13	2.497 ± 0.51	-

*Identification confirmed against reference standard purchased from TCI, Supelco, Alfa Aesar, Arcos organic, or Sigma. Other identifications are tentative. Numbers in the compound field indicate unknowns.

Table 2. Mean concentration (mg/mg dry leaf) ± standard error of methylxanthines (caffeine and theobromine), catechins, gallic acid, and L-theanine in each combination of precipitation and MeJA treatment.

	100% Rainfall		75% Rainfall		50 % Rainfall	
Compound	**Control**	**MeJA**	**Control**	**MeJA**	**Control**	**MeJA**
Caffeine	14.84 ± 0.926	16.04 ± 0.712	16.22 ± 0.467	16.72 ± 1.132	17.92 ± 0.611	16.85 ± 1.124
Theobromine	0.04 ± 0.016*	0.05 ± 0.023*	0.05 ± 0.018*	0.08 ± 0.047*	0.14 ± 0.041*	0.13 ± 0.04*
Catechin	0.84 ± 0.141*	0.55 ± 0.091*	0.42 ± 0.084*	0.48 ± 0.083*	0.47 ± 0.052*	0.37 ± 0.082*
Epicatechin	10.56 ± 1.39	8.47 ± 0.685	7.29 ± 0.697	8.45 ± 1.111	8.16 ± 0.885	7.01 ± 0.787
Epicatechin gallate	10.97 ± 1.042	9.74 ± 1.011	8.72 ± 0.852	8.8 ± 0.645	8.76 ± 0.722	7.63 ± 0.612
Epigallocatechin	20.42 ± 1.746	19.54 ± 1.428	18.49 ± 1.405	20.27 ± 0.727	20.96 ± 1.746	20.82 ± 1.377
Epigallocatechin gallate	37.54 ± 2.565	39.57 ± 2.044	37.56 ± 2.347	36.86 ± 1.205	36.8 ± 2.458	36.39 ± 3.599
Gallocatechin	2.51 ± 0.258	2.15 ± 0.289	2.08 ± 0.196	2.08 ± 0.284	2.13 ± 0.145	2.17 ± 0.364
Gallic acid	0.39 ± 0.258*	0.21 ± 0.214*	0.42 ± 0.272*	-	-	0.18 ± 0.177*
l-Theanine	4.4 ± 0.607	5.68 ± 0.675	5.36 ± 1.188	4.62 ± 1.096†	5.14 ± 0.974	4.86 ± 1.091

*estimated concentration extrapolated from calibration function in all samples, excluded from analysis. †estimated concentration in 8 samples but included in analysis.