MANUEL ANTONIO SOLÍS VARGAS

MUDANÇAS CLIMÁTICAS NA AGRICULTURA: EFEITO DO DEFICIT HÍDRICO SOBRE O MECANISMO DE RESPOSTA DO TOMATEIRO À ARTRÓPODES HERBÍVOROS E À SUA PRODUTIVIDADE

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-graduação em Entomologia, para obtenção do título de *Doctor Scentiae*

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APROVADA: 9 de abril de 2018.

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DEDICATÓRIA

A minha Mãe e minha Filha

Ao meu Pai

Aos meus Irmãos, Irmãs, Sobrinha, Sobrinhos

A minha família que sempre me apoia

Aos meus Amigos e Colegas

Aos meus Professores e Mestres

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A vida, Deus e as forças da natureza que tem me levado pelo caminho certo.

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BIOGRAFIA

Manuel Antonio Solís Vargas, filho de Victor Manuel Ramón Solís Arce e María Gabriela de Jesús Vargas Villalobos, nasceu no dia 27 de setembro de 1979 em São José da Costa Rica.

Em 1998 iniciou o curso de Manejo e Proteção dos Recursos Naturais pela Universidade Estadual a Distancia da Costa Rica (UNED) graduou-se como licenciando no ano 2008.

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ABA: Abscisic Acid

ACC: Aminocyclopropane-1-Carboxylic Acid

ANOVA: Analisys of Variance

CaCl$_2$: Calcium Chloride

ET: Ethylene

ETc: Crop Evapotranspiration

ETo: Reference Evapotranspiration

HCl: Hydrochloric Acid

IAA: Indole-Acetic Acid

JA: Jasmonic Acid

L-BApNA: N-α-Benzoil-L-arginine 4-nitroanilide hydrochloride

LC-MS: Liquid Chromatography Mass Spectrophotometer

LOX: Lipoxygenases

MeJa: Methyl Jasmonate

MRM: Multiple Reaction Monitoring

PPO: Polyphenol oxidases

PI: Proteinase inhibitor

RH: Relative Humidity

RIN: Real Irrigation Needed

SA: Salicylic Acid

SM: Supplemental Material

UHPLC: Ultra High-performance liquid chromatography

RESUMO

SOLIS-VARGAS, Manuel Antonio. D.Sc. Universidade Federal de Viçosa, abril de 2018. **Mudanças climáticas na agricultura: Efeito do deficit hídrico sobre o mecanismo de resposta do tomateiro à artrópodes herbívoros e à sua produtividade.** Orientadora: Maria Goreti de Almeida Oliveira. Coorientadores: Carlos Nick Gomes, Marcelo Coutinho Picanço, Ângelo Pallini Filho, Gláucia Cordeiro.

A água media as interações planta-praga afetando a produtividade e o sucesso reprodutivo dos organismos. As interações entre insetos e suas plantas hospedeiras são afetadas pela especialização na alimentação das espécies herbívoras, geralistas ou especialistas, assim como a disponibilidade de água. O objetivo deste trabalho foi comparar as respostas defensivas do tomateiro pela sinalização dos hormônios vegetais e compostos de defesa pela via das lipoxigenases, quando presentes estresses tanto biótico como a herbivoria por ácaros e insetos, assim como abiótico de água, especificamente o deficit hídrico, de forma simultânea, no campo aberto. Propõe-se responder: Como esses fatores podem afetar a produtividade do tomateiro e a susceptibilidade a insetos que broqueiam os frutos?; O deficit hídrico afeta as defesas induzidas no tomateiro, a expressão de hormônios vegetais e os compostos de defesa pela via das lipoxigenases e polifenol oxidases?; e se em combinação com a herbivoria as expressões de hormônios vegetais e compostos de defesa, podem aumentar ou diminuir?; também se o deficit hídrico afeta o desenvolvimento e sucesso reprodutivo de insetos pragas como a traça do tomateiro, *Tuta absoluta* (Lepidoptera: Gelechiidae). Para isto foi desenhada uma parcela experimental de tomate, onde um setor da parcela foi aplicado uma irrigação idônea, sendo do 100 % de demanda de água da cultura. No outro setor da parcela foi aplicado uma irrigação do 50% de demanda da cultura. Um desenho experimental de três fatores foi aplicado para os analises estatísticos. Cada setor da parcela composto de cinco linhas (blocos) com plantas nas que foram aplicados 10 tratamentos ao acaso, sendo cada planta uma unidade amostral. Os tratamentos consistiram de plantas sem infestar e infestadas com ácaros herbívoros especialistas, *Tetranychus evansi* e geralistas *T. urticae* (Acari: Tetranychidae) além de lagartas especialistas, *T. absoluta,* de forma isolada e em combinações das três espécies. Também, outras seis plantas de cada setor foram selecionadas para determinar taxas de oviposição e mortalidade de *T. absoluta*. Uma folha de cada planta foi coberta com uma sacola de organza e as mariposas liberadas dentro. Das folhas do tomateiro foram determinadas, as concentrações de hormônios vegetais e aminoácidos, também a atividade de lipoxigenases, polifenol oxidases e a concentração de inibidores de proteinase. Os frutos maduros foram classificados quanto ao tamanho, peso e danos pela broca-pequena do tomateiro *Neoleucinodes elegantalis* (Lepidoptera: Crambidae). Também, foram avaliadas as taxas de oviposição, e mortalidade da traça do tomateiro *T. absoluta* em plantas que tiveram irrigação idônea e plantas em deficit hídrico. Os perfis dos compostos bioquímicos mostram correlações positivas e negativas, indicando várias vias de sinalização e respostas defensivas mediadas pelos hormônios. O rendimento do tomateiro foi afetado pelo deficit hídrico, mas não pela herbivoria, podendo tornar as culturas mais suscetíveis a *N. elegantalis*. Interações entre as cascatas hormonais foram mediadas pelo deficit hídrico e a herbivoria, induzindo diferentes expressões dos compostos de defensa. O ácaro rajado *T. urticae* teve uma influência importante na ativação das lipoxigenases, polifenol oxidases e indução de inibidores de proteases do tomateiro, de forma tanto negativa como positiva. O ácaro vermelho *T. evansi* e a traça do tomateiro *T. absoluta,* marcaram principalmente, respostas cruzadas dos hormônios quando compartilharam plantas hospedeiras com outros herbívoros. O tomateiro sob deficit hídrico foi menos adequado para o desempenho de *T. absoluta*, devido as defesas mediadas pelo ácido jasmônico e compostos induzidos por herbivoria com alta produção de inibidores de proteases, coincidindo com a “hipótese da planta vigorosa”. As sinalizações cruzadas dos hormônios vegetais no tomateiro, mostraram grandes diferenças entre as condições hídricas e a herbivoria por *T. absoluta*. Conclui-se que, o tipo de mecanismo de alimentação e especialização do herbívoro foi um fator importante na variação destas interações. Assim como a quantidade de espécies que podem-se alimentar numa planta simultaneamente. A vias das lipoxigenases foi ativada com a produção de compostos de defesa, e o deficit hídrico influi na direção da atividade das lipoxigenases e polifenol oxidases.

ABSTRACT

SOLIS-VARGAS, Manuel Antonio. D.Sc. Universidade Federal de Viçosa, April, 2018. **Climate change in agriculture: Hydric deficit effect on the defense mechanism response of tomato to herbivore arthropods and it productivity.** Advisor: Maria Goreti de Almeida Oliveira; Co-advisors: Carlos Nick Gomes, Marcelo Coutinho Picanço, Ângelo Pallini Filho and Gláucia Cordeiro.

Water mediates plant-pest interactions affecting the productivity and reproductive success of organisms. Interactions between insects and their host plants are affected by the feeding mode of herbivorous species, generalists or specialists, as well as the availability of water. This work aimed to compare the defensive tomato responses by the phytohormones signalling and defense compounds of the lipoxygenases pathway, under biotic stress as herbivory by mites and insects, as well as water abiotic stress, specifically water deficit, simultaneously in the open field. It was proposed to answer: How can these factors affect tomato productivity and susceptibility to fruit-borer insects?; The water deficit affects the tomato induced defenses, as the phytohormones expression and the defense compounds through the lipoxygenases and polyphenol oxidases pathways?; and if in combination with herbivory can the expressions of plant hormones and defense compounds increase or decrease?; also if the water deficit affects the development and reproductive success of insect pests such as the tomato moth, *Tuta absoluta* (Lepidoptera: Gelechiidae). For this, a tomato experimental plot was designed, where a sector of the plot was applied a suitable irrigation, of 100% the water demand of the culture. In the other sector of the plot, an irrigation of 50% of crop demand was applied. An experimental design of three factors was applied for the statistical analyses. Each plot sector consisted of five lines (blocks) with plants on which 10 treatments were applied randomly, each plant being a sample unit. The treatments consisted of uninfested plants and infested with herbivorous specialist mites, *Tetranychus evansi* and generalists *T. urticae* (Acari: Tetranychidae) in addition to specialist caterpillars, *T. absoluta*, isolated and in combinations of the three species. In addition, six other plants from each sector were selected to determine *T. absoluta* oviposition rates and mortality. One leaf of each plant was covered with an organza bag and the moths released inside. From tomato leaves, the phytohormones, amino acids proteinase inhibitors concentrations, the lipoxygenases and polyphenoloxidases activities were determined. Mature fruits were classified according to size, weight and damage by tomato borer

Neoleucinodes elegantalis (Lepidoptera: Crambidae). Also, the oviposition rates and mortality of the tomato leaf-miner *Tuta absoluta* (Lepidoptera: Gelechiidae) were evaluated, in plants that had adequate irrigation and plants in water deficit. Profile patterns of biochemical compounds show several positive and negative correlations, indicating several hormone crosstalk-signalling pathways mediating defensive responses. Tomato yield were affected by water deficit, but not by herbivory, and could turn crops more susceptible to *N. elegantalis*. The interactions of the hormonal cascades were mediated by water deficit and herbivory, resulted in different expressions of defense compounds. The two-spotted spider mite *T. urticae* had an important influence on the activation of lipoxygenases and polyphenol oxidases and proteinase inhibitors induction, both negative and positive. The red spider mite *T. evansi* (Acari: Tetranychidae) and tomato leafminer *T. absoluta,* mediated phytohormones cross-responses, mainly, when sharing host plants with other herbivores. The tomato under water deficit was less suitable for *T. absoluta* performance, due to the defenses mediated by the jasmonic acid and herbivory induced compounds with high production of protease inhibitors, coinciding with the "plant vigour hypothesis". Cross-talk phytohormones pathways in tomato plants, showed large differences between water conditions and herbivory by *T. absoluta*. It was concluded, that the type of feeding mechanism and specialization of the herbivore was an important factor in the variation of these interactions. As well as the quantity of species that can feed on a plant simultaneously. A lipoxygenases pathway was activated with the production of defense compounds, and the water deficit influences the lipoxygenase and polyphenol oxidases activity.

INTRODUÇÃO GERAL

Uma das espécies hortícolas mais importantes do mundo é o tomate (*Solanum lycopersicum* L), sendo a hortícola mais cosmopolita e amplamente disseminada no mundo, com um aumentado na produção global de mais de 300% nos últimos 40 anos (FAOSTAT, 2018). No Brasil é produzida em quase todos os estados do país. De grande importância econômica, gera empregos em todos os segmentos de sua cadeia produtiva. O fruto é fonte de ácido fólico, vitamina C, potássio e carotenoides, sendo o licopeno (antioxidante) o mais destacado (SILVA *et al,* 2017). Entretanto, não há na agricultura brasileira outra cultura tão complexa, do ponto de vista agronômico e de riscos econômicos tão elevados, em virtude principalmente da infestação por diversas pragas e doenças, tanto na cultura para consumo *in natura*, como para indústria (FILGUEIRA, 2005; GONÇALVES NETO *et al*., 2010).

Em resposta às necessidades de controle de patógenos e pragas, além de deficiências hídricas e melhora das qualidades nutricionais das plantas, tem sido aplicada técnicas de melhoramento genético e de manejo integrado. Entre os artrópodes-pragas, os ácaros podem se tornar um grande problema em condições de seca e elevadas temperaturas (ARAGÃO et al., 2002; MORAES e FLECHTMANN, 2008). O ácaro vermelho (*Tetranychus evansi* Baker & Pritchard, 1960 (Acari: Tetranychidae)), se alimenta do conteúdo celular e é um especialista em solanáceas, principalmente no tomateiro. Este ácaro é capaz de suprimir e contornar as defesas de tomate (ALBA *et al.,* 2015; SARMENTO *et al.,* 2011a), e causa mais danos às folhas e melhora seu desempenho nas folhas de tomateiro submetido ao deficit hídrico e seca (XIMÉNEZ-EMBÚN *et al.,* 2016).

O ácaro rajado (*Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae)), é um herbívoro geralista e um indutor de proteínas de defesa no tomateiro (OLIVEIRA *et al.,* 2016), e tem um desenvolvimento similar em plantas em deficit hídrico e em condições idôneas de irrigação (SADRAS, 1998). Estas espécies de ácaros produzem teias, fato que dificulta a ação de inimigos naturais, além que diminui a eficiência de agrotóxicos e oferece proteção a eles em caso de condições climáticas adversas (GERSON, 1985; SARMENTO *et al.,* 2011b).

Lagartas como a traça-do-tomateiro (*Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)), é um herbívoro especialista em tomateiro, ataca toda a planta em qualquer estádio de desenvolvimento, formando galerias transparentes, principalmente nas gemas apicais, reduzindo a capacidade fotossintética da planta e degrada severamente a produção agrícola. Também perfuram os frutos, que são depreciados para comercialização (SILVA e CARVALHO, 2004; DESNEUX *et al.,* 2010; 2011). Esta lagarta induze as defesas diretas do tomateiro, mas tem um pobre desenvolvimento em plantas sobre estresse hídrico de seca (HAN *et al.,* 2014). Também, a broca pequena do tomate, *Neoleucinodes elegantalis* (Guenée, 1854) (Crambidae: Lepidoptera) causa dano direto aos frutos. Esta espécie é nativa da região Neotropical e foi registrada na América do Sul e Central, onde a perda de produção pode variar de 50% a 90%, não induze compostos de defesa nos frutos do tomateiro (SILVA *et al.,* 2017).

As vias de defesa são ativadas por uma cascata de sinalização em resposta à presença do agente daninho induzindo a expressão ou aumento da expressão de genes envolvidos na defesa (GLAZEBROOK, 2005; MCDOWELL e DANGL, 2000). Os danos causados por herbívoros desencadeiam eventos bioquímicos que resultam na expressão de mecanismos de defesa do tomateiro. Por isto, esta planta tem sido um modelo em estudos das interações herbívoro-planta e expressão de genes que codificam compostos de defesa (CAICEDO e PERALTA, 2013). O tomateiro possui dezenas de compostos secundários provenientes de várias vias metabólicas e os efeitos defensivos destes compostos podem variar em magnitude e direção entre diferentes espécies de herbívoros (AGRAWAL e WEBER, 2015). A correlação entre compostos secundários e herbivoria pode ser em uma direção positiva ou negativa dependendo da especificidade do grupo de herbívoro (ALI e AGRAWAL, 2012). Assim, um composto que é repelente para um grupo de inseto poder ser atraente para outro (AGRAWAL e WEBER, 2015).

A defesa constitutiva é expressa permanentemente pela planta, independentemente de estar sendo atacada ou não. Defesas induzidas estão relacionadas ao aumento do nível de metabólitos secundários ou proteínas relacionadas à defesa, decorrentes do ataque ou danos à planta por artrópodes e patógenos herbívoros. Estás defesas são consideradas defesas diretas, pois diminuem a qualidade nutricional da planta para o herbívoro (KANT *et al.,* 2004). Uma das principais formas de defesa direta das plantas contra o ataque de artrópodes é mediada pela via das lipoxigenases, onde ocorre a produção de hormônios, como o ácido jasmônico que ativa os genes que expressam inibidores de proteases e compostos polifenólicos (MERIÑO-CABRERA *et al,* 2018; SARMENTO *et al*, 2011a).

Metabólitos secundários tem efeitos consideráveis no desenvolvimento ou "fitness" de artrópodes herbívoros, por meio de uma diversidade de modos de ação, provendo defesa para as plantas, incluindo resistência contra herbívoros específicos (AGRAWAL e WEBER, 2015). A ingestão de proteínas de defesa como inibidores de proteases e polifenois, interfere no processo de degradação de proteínas nos artrópodes. Os inibidores são considerados agentes anti-metabólicos, pois levam os herbívoros a uma deficiência proteica. Essa interferência na digestão diminui a disponibilidade de aminoácidos, prejudicando a síntese de proteínas necessárias ao crescimento, desenvolvimento e reprodução dos artrópodes herbívoros (OLIVEIRA *et al.,* 2005; PILON *et al.,* 2006; SCOTT *et al.,* 2010; SARMENTO *et al.,* 2011).

Plantas com danos prévios por herbívoros, podem-se tornar mais susceptíveis a ataques de segundos herbívoros (SAUGE *et al.,* 2006; POELMAN *et al.,* 2008). Por exemplo plantas de tomate infestadas com *T. evansi* produzem uma quantidade inferior de inibidores de proteases do que as plantas danificadas por *T. urticae* (SARMENTO *et al,* 2011a). *Tetranychus urticae* apresenta melhor desenvolvimento e oviposição quando se alimenta de plantas de tomate previamente atacadas por *T. evansi*, mas o dano causado por *T. urticae* ativa a produção de inibidores de proteases, inclusive na presença de *T. evansi* afetando a reprodução e desenvolvimento de ambas espécies (OLIVEIRA *et al,* 2016; SARMENTO *et al,* 2011).

Alguns autores sugerem que metabólitos secundários são de menor importância na defesa de plantas contra os herbívoros em comparação a características morfológicas e de história de vida e que estes químicos secundários não são tipicamente preditivos de herbivoria (LORANGER *et al.,* 2012; SCHULDT *et al.,* 2012). Entretanto, AGRAWAL e WEBER (2015) acham que as interpretações destes resultados podem atrapalhar a correlação de compostos secundários e herbivoria no contexto de múltiplos herbívoros atacando uma mesma planta. O dano simultâneo de patógenos e herbívoros com distintos mecanismos de alimentação, podem gerar diferentes expressões de defesa da planta pela via de sinalização dos hormônios vegetais dos ácidos salicílico e jasmônico, afetando de igual forma o desenvolvimento dos herbívoros (THALER *et al.,* 2002; KAWAZU *et al.,* 2012).

Além dos fatores bióticos citados anteriormente, os fatores abióticos como precipitação e temperatura podem afetar tanto a produtividade como o sucesso reprodutivo dos organismos e as interações entre estes (SILVA *et al,* 2016a; b).

Diversas atividades antrópicas e industriais têm colaborado para aumento nas concentrações de gases de efeito estufa na atmosfera. Esta situação tem mudado o clima global e elevou a temperatura da superfície da Terra em 0,74ºC no século passado (IPCC, 2012). As mudanças climáticas interferem na capacidade dos organismos terrestres, das populações, das comunidades e dos ecossistemas em lidar com as alterações para que consigam ampliar suas respostas desde o nível molecular até o da biosfera (PEÑUELAS *et al,* 2013).

Estudos de modelagem, juntamente com as projeções estatísticas, preveem para longo prazo cenários acometidos pelos impactos das mudanças climáticas e indicam mudanças também nos rendimentos das produções das culturas (ELITH e LEATHWICK, 2009; MAGUIRE *et al,* 2015; SILVA *et al,* 2016a; b). As culturas são mais vulneráveis às alterações climáticas e sofrerão principalmente com a alta taxa de evapotranspiração devido ao aumento da temperatura atmosférica e à falta de água disponível no solo.

Essa combinação de fatores negativos dificultará o desenvolvimento do ciclo biológico das culturas, diminuindo a produtividade, como foi observado para o trigo e a soja (OSBORNE *et al,* 2012). Períodos prolongados de baixa ocorrência de precipitação ou exposição a temperaturas elevadas produzem desequilíbrio nos sistemas ecológico e hidrológico e têm impactos severos na produção agrícola (KARNIELI *et al,* 2010; BOARD e KAHLON, 2011). As áreas com clima idôneo hoje, para a cultura do tomate, irão transformando-se em áreas inadequadas nos próximos 50 a100 anos, devido ao aumento da temperatura e sequia (SILVA *et al.* 2016b)

Assim, as mudanças climáticas podem interferir na produção agrícola e também nas técnicas de manejo do solo, das plantas daninhas e das pragas (HAN *et al,* 2014; SHABANI *et al*, 2012; SILVA *et al,* 2016a). Além disso, impactos indiretos nas culturas, por meio das alterações das populações de fitófagos, dos inimigos naturais e dos patógenos, aumentaram suas incidências e severidades, e dentre estas populações, as pragas estão entre os primeiros organismos a sofrerem com estes fatores climáticos no que se refere à quantidade de indivíduos (HAN *et al,* 2014; GORNALL *et al,* 2010; XIMÉNEZ-EMBÚN *et al,* 2016).

As células vegetais apresentam alta capacidade de resposta a diversos estresses (bióticos e abióticos) por meio de uma rede de resposta flexível e finamente balanceada que envolve componentes como vias de sinalização de redução-oxidação (redox), hormônios do estresse e reguladores de crescimento (FOYER *et al.,* 2016). As vias de sinalização estão associadas a fenômenos de tolerância cruzada, nos quais a exposição a um tipo de estresse aumenta a resistência das plantas a outros estresses bióticos ou abióticos (PASTORI e FOYER, 2002).

Poucos estudos têm descrito como as plantas respondem a través de diferentes vias de sinalização e como as interações cruzadas das cascatas hormonais podem estar sendo mediadas por fatores bióticos e abióticos que afetam a produtividade do tomateiro, ainda menos em condições de campo aberto. Dado isto, o objetivo deste trabalho foi comparar as respostas defensivas do tomateiro pela sinalização dos hormônios vegetais e compostos de defesa da via das lipoxigenases, quando presentes estresses tanto biótico, como a herbivoria por ácaros e insetos; assim como abiótico de agua, especificamente o deficit hídrico; de forma simultânea, no campo aberto. Propõe-se responder:

1. Como esses fatores podem afetar a produtividade do tomateiro e a susceptibilidade a insetos que broqueiam os frutos?;
2. O deficit hídrico afeta as defesas induzidas no tomateiro, a expressão de hormônios vegetais e os compostos de defesa pela via das lipoxigenases e polifenol oxidases?;
3. Em combinação com a herbivoria e deficit hídrico, as expressões de hormônios vegetais e compostos de defesa, podem aumentar ou diminuir?;
4. O deficit hídrico afeta o desenvolvimento e sucesso reprodutivo de insetos pragas como a traça do tomateiro, *T. absoluta*?

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CAPÍTULO I: Hydric deficit and multiple herbivory mediate crosstalk tomato hormones, plant protein defenses and yield in an open field crop

Abstract

Imbalance in ecological and hydrological systems have severe impacts on agricultural production. Water availability is an important factor that can affect productivity and reproductive success of plants and herbivorous. Interactions between herbivorous arthropods and their host plants are affected by feeding specialization, herbivore species and water availability. Here, we try to answer if the water deficit affects the tomato induced defenses, as the phytohormones expression and the defense compounds through the lipoxygenases and polyphenol oxidases pathways. Also, if water deficit in combination with herbivory can affect the expression of tomato hormones and defense compounds can increase or decrease. Furthermore, how these factors can affect tomato productivity and susceptibility to fruit-borer insects. Tomato crop experimental plots with normal and deficit water input environments were stablished in a factorial design with five block lines each and ten plants per line. Two control treatments (before and after infestation), plants infested only with *Tetranychus evansi, T. urticae* or *Tuta absoluta* and the four combinations between these three species. Plant phytohormones and amino acids concentrations, activities of lipoxygenases, polyphenol oxidases and concentrations of proteinases inhibitors were determined. Ripped fruits were classified by size, weighted and the damage by caterpillar borer *Neoleucinodes elegantalis* were determined. Profile patterns of different biochemical compounds show several positive and negative correlations indicating marked several crosstalk-signalling pathways and hormone mediated defensive responses. Tomato yield were affected by water deficit but not by herbivore infestation. Water deficit and herbivory mediated hormonal cascades crosstalk compared to well-watered plants, resulting in different expressions of defensive enzymatic compounds. The generalist spider mite *T. urticae* had a noticeable influence on biochemical tomato compounds in negative (Proline, Ethylene, Lipoxygenases and Polyphenol oxidases) as positive (Zeatin, Indole-Acetic, Jasmonic and Salicylic acids) induction. The specialist tomato herbivores *T. evansi* and *T. absoluta* mark most crosstalk responses when sharing host plants with other herbivores than alone. Water deficit affected tomato yield without influence of herbivory and could turn crops more susceptible to tomato borers.

Keywords; Irrigation, Herbivory, Lipoxigenases, Polyphenol oxidases, Proteinase Inhibitors.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most economically important crop species globally and features as a model organism in many research studies (Caicedo and Peralta 2013; Silva et al. 2016a). The global production has increased more than 300% in the last 40 years (FAOSTAT 2018) and crops in open fields are more influenced by climatic factors (Heuvelink 2005). Statistic projections suggest that areas with optimal climate nowadays will progressively become unsuitable for tomato crop in the next 50 to 100 years due to heat and drought increase (Silva et al. 2016b).

These abiotic factors, especially drought, also affect arthropod pest distributions, biology and interaction with host-plants (Han et al. 2014; Silva et al. 2016b; Ximénez-Embún et al. 2016) The presence of indirect impacts on crops through changes in phytophagous populations, natural enemies and pathogens has been observed (Gutbrodt et al. 2011; Huberty and Denno 2011). These changes have increased their incidence and severity, and among these populations, pests are among the first organisms to suffer from these climatic factors with regard to the number of individuals (Gornall et al. 2010; Ximénez-Embún et al. 2016).

Water-availability mediated interactions between herbivorous insects and their host plants depend on various factors including herbivore feeding specialization, herbivore species and the seasonality of water availability (Han et al 2014; Huberty and Denno 2011). In addition, drought may influence plant chemical defense level leading to either enhanced or reduced resistance to herbivores (Gutbrodt et al. 2011; Inbar et al. 2001; Valim et al. 2016).

Plant cells display a high capacity to respond to diverse stresses (biotic and abiotic) through a flexible and finely balanced response network that involves components such as reduction-oxidation (redox) signalling pathways, stress hormones and growth regulators (Foyer et al. 2016). Signalling pathways are associated with cross-tolerance phenomena in which exposure to one type of stress enhances plant resistance to other biotic or abiotic stresses (Pastori and Foyer 2002).

Important tomato pests as *Tetranychus evansi* (Baker & Pritchard, 1960) (Acari: Tetranychidae), a specialist cell content feeder on solanaceas, is capable of supress and circumvent tomato defences (Alba et al. 2015; Sarmento et al 2011), and causes more leaf damage and enhance their performance on leaves of drought stressed tomato plants (Ximénez-Embún et al. 2016). This, inducing plant protein defenses and increasing phagostimulants amino acids. Therefore a very close but generalist spider mite *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) is a great inductor of plant protein defenses (Oliveira et al. 2016) but performing equally on drought stressed and well-watered cotton (Sadras 1998). On the other hand, the leafminer specialist *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) poorly performs on drought stressed tomato (Han et al. 2014).

Few of these studies have look how plants respond through signalling pathways and crosstalk hormone cascades can be mediated by biotic and abiotic factors affecting tomato crop yields, much less in open field experiments. So, we goal to determine if the water deficit affects the tomato induced defenses, as the phytohormones expression and the defense compounds through the lipoxygenases and polyphenol oxidases pathways. Also, if water deficit in combination with herbivory can affect the expression of tomato hormones and defense compounds can increase or decrease. Furthermore, how these factors can affect tomato productivity and susceptibility to fruit-borer insects.

MATERIAL AND METHODS

Tomato crop

Tomato (*Solanum lycopersicum* L.) of Thaíse cultivar from Feltrin seeds (Rio Grande do Sul, Brazil) was used. This cultivar present tolerance and resistance to tomato mosaic virus, tomato yellow leaf curl virus, *Fosarium oxysporum* Schltdl. (Nectriaceae; Sordariomycetes), *Verticillium dahliae* Kleb (Plectosphaerellaceae; Sordariomycetes) *Meloidogyne arenaria* (Chitwood, 1949), and *M. incognita* (Kofoid & White, 1919) (Heteroderidae; Secernenta). Seeds were planted in plastic trays (8 x 16 cells) with commercial substrate, covered with plastic film and maintained into a germination chamber at 30ºC for three days. After germination, trays were left in a greenhouse with automatic irrigation system. A fertigation were applied twice until transplantation.

After 30 days, seedlings were transplanted to soil previously treated and fertilized in a field area of the Plant Science Department of the Federal University of Viçosa (UFV), Minas Gerais, Brazil. An irrigation system by drip hosepipe and a tutoring vertical system were installed (see supplemental material; SM1). Tomato crop consisted in two plots of six lines each, containing 14 plants spaced 0.6 m between them and 1 m between lines. Normal irrigation were maintained for two weeks and controlled irrigation were then applied to both plots. One of them with normal water supplied considered as 100% irrigation for ideal crop management and production, it means a proportion of 8L/m^2/day and 3L/plant/day (Heuvelink 2005; Silva and Vale 2007). In the other plot a restricted irrigation from 50% to 25% of normal irrigation was applied without compromising the plants development.

A protective spraying (see supplemental material; SM5) was applied twice per week for one and a half month since transplantation and then stopped before experimental treatments was performed. Fertigation, weeding and stripping crop management were executed for ideal production using local procedures based on standard agronomic practices (Alvarenga 2013). When pesticides effect passed and the crop irrigation treatment were stabilized to 100% and 50% irrigation, the infestation experiment were performed.

Meteorological measurements from a field station (IrriPlus E5000) were downloaded and inserted in an Excel (Microsoft Office ®) spreadsheet for irrigation time be determined every two to three days. Soil samples at 40 cm deep were taken with a manual auger. Soil humid weight registered and then dried in a lab's stove for 24 hour at 100 ºC. The dry weight were register and the differences between plots compared (see supplemental material; SM2). The Real Irrigation Needed (RIN) were determine by estimating the Crop Evapotranspiration (ETc) using adjustment coefficients over the Reference Evapotranspiration (ETo) of tomato combined with meteorological, soil, water, plant and equipment data on the Penman-Moteith-FAO56 model (Allen et al. 1998; Delazari et al. 2016; see supplemental material; SM3 for equations).

Herbivores infestation and experimental design

The spider mites (*T. evansi* and *T. urticae*) were obtained from the Acarology Laboratory and adults of *T. absoluta* from the Integrated Pest Management Laboratory, both of the Entomology Department, UFV. New populations were started and raised in a controlled environment in the Insect Laboratory of the Biochemichal and Molecular Biology Department, UFV, on tomato leaves of the cultivar Santa Clara I-5300 at 25 ± 5ºC, R.H. of 70 ± 5% and photoperiod of 12h.

The arthropods were collected and transferred to the plants in the filed plot for infestation. Ten treatments were applied (counting five plants per treatment) randomly distributed in each plot line (five lines per plot), so experimental design consisting on two plot sectors (50% and 100% of irrigated for culture demand). Each sector were divided in five block lines containing ten differently treated plants. Border lines were also added to the plot and one or two plants on each extreme line were left to avoid border effects.

Treatments consist on: non-infested plants before infestation (CBI); non-infested control plants after infestation (CAI); plants infested only with 100 *T. evansi* female adults; with only 100 *T. urticae* female adults; or with only nine *T. absoluta* caterpillars. Also, plants that were applied a mechanical damage using fine forceps to prick the selected leaves; plants infested with 50 *T. evansi* + 50 *T. urticae*; plants infested with 100 *T. evansi* + 9 *T.absoluta*; plants infested with 100 *T. uticae* + 9 *T.absoluta;* and plants infested with 50 *T. evansi*, 50 *T. urticae* and 9 *T. absoluta*, at the same time. The fourth bottom up full-developed leave of each plant were selected and infested for seven days until sample collecting.

Plant physiological and biochemical defense response

Sample collecting

The leaflets from treated leaves were cut with scissors and put inside aluminum foil envelops immediately bathed with liquid nitrogen inside a Styrofoam box and rapidly toke to a -80 ºC freezer.

Phytohormones and Proline

For extracts preparation the tomato leaflets were macerated in liquid nitrogen and the tissue weighted. For each 100 mg of tissue 200 µL of the extractive solution (methanol 20% / Isopropanol 79% / Acetic Acid 1%) subjected to ultrasound for 30 min and centrifuged at 14000 *g* for 30 min at 4 °C. The supernatant was collected, and the procedure repeated. The extracts were stored at -80 °C until analysis in the LC-MS of type UHPLC QqQ been performed.

Approximately 300 µL of the extracted samples were filtered and placed in vials, then 5.0 µL of the extracts were injected into the LC / MS system of the Nucleus of Biomolecules Analyzes (NuBioMol) of Helath and Biological Sciences Center of UFV. It was used a chromatographic column (Agilent Eclipse Plus, RRHD, 1.8 um, 2.1x50 mm) with flow of 0.3 mL / min coupled online to a triple quadrupole mass spectrometer QqQ (Agilent). The phytohormones analyzed were Abscisic Acid (ABA), Jasmonic Acid (JA), Methyl Jasmonate (MeJa), Salicylic Acid (SA), 1-Aminocyclopropane-1-Carboxylic Acid (ACC-Ethylene), Indole-3-Acetic Acid (IAA), Zeatin and the amino acid Proline. The mass spectrometer operated by alternating positive and negative mode according to retention time for each hormone and monitoring the sample in MRM mode (*multiple reaction monitoring*) to allow the hormones detection.

The assays were performed with five biological replicates of each treatment. The mass spectra generated were processed using the MassHunter (Agilent Technologies) software to obtain the chromatograms extracted (XIC) of each transition and to obtain the area values as indicative of the abundance of each hormone. Area values were adjusted using Skyline sortware (MacCoss Lab; University of Washington) and exported to Excel spreadsheet. A standard curve of each hormone, varying concentrations of 0.1 to 300 ng / mL, was used to convert the area values of the identified metabolites into concentration values expressed as (ng of hormone / g of leaf tissue; see SM3 for equation).

Enzymatic activities

The plant material of each plant was weighed in analytical balance, macerated in liquid nitrogen, transferred to Sodium Phosphate buffer solution 0.05M pH 6.5 (lipoxygenases analysis) or buffer solution Tris-HCl 0.1M, pH 8.2 with 20mM of $CaCl_2$ (proteinase inhibitors analysis); 1:3 (w/v). The extracts were centrifuged at 17200 *g* by 60 minutes at 4°C (Meriño-Cabrera et al. 2018) and the supernatant used for the determination of the enzymatic activities. The protein concentration was determined from the extracts using bovine serum albumin (BSA; 0-0.2mg/mL) (Bradford 1976). Enzymatic activities were determined using a Hitachi Ratio Beam Spectrophotometer U-5100 of the Enzymes, Proteins and Peptides Biochemical Laboratory of the Institute of Biotechnology Applied to Agriculture (BIOAGRO, UFV).

Lipoxygenases

The activity of lipoxygenases (LOX) on linoleic acid was determined (Axelrod et al. 1981), in a mixture of 10 µL of the leaf extract and 20 µL stock solution of sodium linoleate in 1000 µL of sodium phosphate buffer. The absorbance of the reaction mixture was determined every 30 seconds at 234 nm for 150 seconds. The final absorbance values resulting from differences between the absorbance at 150 and 30 seconds. The specific activity of product formation were calculated from the final absorbance values in (µmol.s^{-1}) / (mg/mL) using the molar extinction coefficient of 25000M^{-1} cm^{-1}. (see SM3 for equations).

Proteinase Inhibitor

Proteinase inhibitor (PI) concentrations in the foliar extract were determined by inhibition of the comercial trypsin activity acting with the foliar extract on substrate N-α-Benzoil-L-arginine 4-nitroanilide hydrochloride (L-BApNA) 1,2 mM (Erlanger et al. 1961). The control of purified trypsin had 100 µL of trypsin solution 4.7 x 10 $^{-5}$ M in micro tube with 1100 µL of buffer Tris-HCl and 500 µL of subtrate. The samples had a micro tube with 500 µL buffer that received 100 µL of foliar extract and 100 µL of trypsin solution. After five minutes of incubation, this solution was transferred to another micro tube with 500 µL buffer and 500 µL substrate. The absorbance of the test and control solutions was determined at 410 nm every 30 seconds for 150 seconds of reaction. The final absorbance were calculated by the differences between the absorbance at 150 and 30 seconds. The results were converted to mg of trypsin inhibited per gram of protein (Kakade et al. 1974; see SM3 for equation).

Polyphenol oxidases

The polyphenol oxidases (PPO) activity was determined using catechol-like substrate (Soliva et al., 2000). Each reaction consisted of 960 µL of the catechol 0.2 M and 40 µL of the plant extract. The product formation was calculated at 410 nm over a 150 seconds period with 30 seconds intervals. The final absorbance were calculated by the differences between the absorbance at 150 and 30 seconds. The activity of the enzyme was measured in (µmol.s^{-1}) / (mg/mL) using the molar extinction coefficient 1470M^{-1} cm^{-1} (SM3 for equation).

Tomato production

Tomato ripe fruits crops were evaluated by fruit quantity, size and weight. Fruits where classified into three diameter categories 10, 8, 6.5 and 5 cm, per plant. Then, these fruit groups were weighted in an analytical balance and averaged. Besides, fruit damage by the small tomato borer caterpillar *Neoleucinodes elegantalis* were determined.

Statistical analyses

All groups of data, of each plot were firstly analyzed by a Three-way ANOVA to determine that there were not block (lines) effects of the experimental design. Then a Two-way or One-way ANOVA proceed follow by pairwise comparisons of means with Student-Newman-Keuls Method at 5% significance using SigmaPlot 12 (Systat Sofware, Inc.).

Due to high variation coefficients of enzymatic groups of data, these were analysed by linear mixed effects models (LME, package nlme of R, Pinheiro 2014) with a Poisson error distribution (log link function). Contrast among mean treatments were determined by the general linear hypothesis testing (function glht of the package lsmeans in R, Lenth 2016) (R Core Team, 2017). To elucidate more deep comparisons the Spearman Rank Order Correlation method were applied to all biochemical groups of data.

RESULTS

Plant physiological and biochemical defense response

Phytohormone abscisic acid (ABA) and amino acid Proline associated to water deficit showed significant differences between plots, herbivore infestation and interaction between these two factors (Table 1.1). Elevated concentrations of ABA and Proline were found in plants on deficit-watered plots (Figure 1.1).

For ABA, control plants before experiment implementation show the highest concentrations, so ABA concentration seems that decreased with time. This phytohormone concentration were affected by infestation treatments only on deficit-watered plants, especially those infested with *T. urticae* (Figure 1.1a). For Proline there were no differences between treatments on deficit-watered plot, but in plants under normal-watered environment the control plants before experiment implementation also have the highest concentrations and treatments with *T. urticae* show the lowest concentrations (Figure 1.1b).

Phytohormone ACC-Ethylene showed significant differences between variables, but not for the interaction between factors (Table 1.1). This phytohormone had higher concentrations in control plants before experiments, seeming a time response. However, it did not respond to water deficit in any treatment and their concentration were lower in plants under deficit-watered environment in most treatments. Contrarily on normal-watered plants, it was induced by *T. evansi*, *T. absoluta* and mechanical damage (Figure 1.2a).

The phytohormone IAA showed no difference between water regimes, but there were differences in herbivory treatments and the interaction between these factors. It means that at the overall data when comparing both irrigation conditions, these have no differences, but it has between some pairwise treatments (Table 1.1; Figure 1.2). Normal-watered plants did not show differences in IAA concentrations between treatments and deficit-watered only in plants infested with *T. urticae*. Plants infested with *T. evansi*, *T. urticae* and mechanical damage showed differences between water regimes (Figure 1.2b).

Zeatin concentration showed a similar pattern to IAA with significant differences between treatments and the interaction between factors (Table 1.1). These two phytohormones were highly induced exclusively by *T. urticae* under water deficit. These plants together to those infested with *T. evansi* plus *T. absoluta* and those infested with the three species showed differences between irrigation environments (Figure 1.2b).

The concentrations of jamonates defense phytohormones showed very irregular profiles. Concentrations of Jasmonic and Salicylic acids were significantly between plots, treatments, and the interaction between both factors, showing dependent effects on these phytohormones concentration in tomato plants. Oppositely, the Methyl-Jamosnate profile showed any factor difference (Table 1.1).

The JA had higher concentrations in plants under normal-watered environment in most treatments. On plants under normal-watered environment JA concentration were significantly induced on all infested plants especially those having a combination of *T. absoluta* with each spidermite specie and by mechanical damage. On plants under deficit-watered environment JA concentrations were induced in plants infested with *T. urticae* and in combination with *T. absoluta* (Figure 1.3a).

The SA concentrations were higher in plants under deficit-watered environment and were induced in no-infested control plants and all infested ones. SA were highly induced in plants infested with *T. urticae* followed by plants infested with *T. urticae* plus *T. absoluta* and the three species. Plants under normal-watered environment did not show differences between treatments (Figure 1.3b).

Lipoxygenases (LOX), Proteinase inhibitors (IP) and Polyphenol oxidases (PPO) were significantly different between plots, treatments, and the interaction between both factors, showing dependent effects of these compounds in tomato plants. Lipoxygenases profile were very irregular and only in three treatments showed differences between plants under irrigated environments, but with tending to normal-watered plants had higher LOX activities. Which were induced by mechanic damage and the three species feeding simultaneously on plants on normal-watered plants. Meanwhile in deficit-watered plants seemed to be induced only in the presence of *T. urticae* (Figure 1.4a).

The Proteinase Inhibitors profile showed a more regular pattern with most of plants under deficit-watered environment having higher concentrations of PI, except for those infested with the three herbivores simultaneously. In plants on normal-watered environment, the PI concentrations were induced in all treated plants except in those infested with both spidermite species and with *T.urticae* plus *T.absoluta*. Aversely, no-infested control plants under water deficit induced PI concentrations equally than plants infested with one exclusively herbivore specie. However, plants infested with two or three herbivore species decreased PI concentrations (Figure 1.4b).

The Polyphenol Oxidases profile were more similar to it of LOX, showing higher concentrations on normal than deficit-watered plants except in plants infested with both spider mite species. Normal-watered control plants showed high PPO activity that seems had been supressed by herbivore damage, except in plants infested with the three arthropod species. Contrary to deficit irrigated plants in which PPO activity were apparently induced in plants infested with *T. urticae*, *T. absoluta* both spidermite species and the simultaneous damage of the three herbivores (Figure 1.4c).

Overall correlations between tomato biochemical compounds show many positive as negative cross-talk interactions (Table 1.3). Highlighting the positive correlation between LOX, PPO and JA, but negative of these three with SA, ABA and

Proline. In addition, ACC were positive with LOX and IAA. PI showed any correlation with JA, but positive correlations with Zeatin and SA, and these three a negative with ACC. The ABA, SA and Proline also showed positive correlations. It were found more correlations in the normal than deficit-watered plants. Six positive correlations (LOX/PPO; PI/Zeatin; JA/SA; SA/IAA; JA/IAA; ACC/Proline) in the normal-watered plants (Figure 5). Two positive (LOX/PPO; PI/Zeatin) and two negative (PI/ACC; JA/Proline) in the deficit-watered ones (Figure 1.6).

Crop production

Ripped tomato fruits two-way ANOVA showed differences only between water regimes, but not for herbivory infestations or factor interactions (data not showed). Therefore, only the irrigation environments had effects on fruit production affecting quantity, size and weight of ripped fruits and susceptibility to the small borer caterpillar (*N. elegantalis*). Plants under water deficit produce more quantity of fruits with lower size and weight, besides been more exposed to the small borer caterpillar, as well (Table 1.4).

DISCUSSION

Water availability is an important factor that can affect both the productivity and reproductive success of organisms and the interactions between them (Gutbrodt et al. 2011; Inbar et al. 2001). Prolonged periods of low occurrence of precipitation or exposure to high temperatures produce an imbalance in ecological and hydrological systems and have severe impacts on agricultural production (Board and Kahlon 2011; Karnieli et al 2010; Kogan et al. 2013). Water-availability mediate interactions between herbivorous insects and their host plants are affected by various factors including herbivore feeding specialization, herbivore species and the seasonality of water availability (Han et al 2014; Huberty and Denno 2011). In addition, hydric deficit may influence plant chemical defense levels leading to either enhanced o reduced resistance to herbivores (Gutbrodt et al. 2011; Inbar et al. 2001).

Both herbivore feeding and water availability affected tomato physiological and biochemical defense compounds. Tomato phytohormone ABA and amino acid Proline had higher concentrations in plants under water deficit and feeding by spider mites. For ABA the spider mites presence seemed to increase its concentration in stressed plants and for Proline the presence of *T. urticae* reduced it in non-stressed ones.

ABA positively respond to water deficit due to control of stomatal movement and leaf senescence (Liang et al 2014; Nguyen et al. 2016). In addition, a deficiency of this phytohormone would take tomato plants into an herbivory susceptibility increase (Thaler and Bostock 2004). Proline in the other hand acts as a cell osmoprotectant (Suzuki et al 2014), so it is a good water deficit indicator. However, at the same time acts as stimulant for cell content feeders as *T. evansi* (Ximénez-Embún et al. 2016) increasing plant susceptibility. Therefore, plants may reduce Proline concentrations to reduce leaves palatability.

The ACC-Ethylene concentration showed a decrease from before to after the experiment period and were lower in deficit-watered plants without responding to herbivore feeding. However, induced by mechanical, *T. evansi* and *T. absoluta* damage in normal-watered plants, which also means that this phytohormone did not respond in any plant infested with *T. urticae*. The ACC-Ethylene is a modulator of herbivore-induced responses in plants and the major inducer of leaf senescence (von Dahl and Baldwin 2007; Kim et al. 2015), so it can mediate JA defences (Nguyen et al 2016) and is an antagonist of ABA response (Tanaka et al 2015). Thus, ABA and ACC tend to show and negative correlation, even without been significant.

The concentration of IAA phytohormone were significantly higher in deficit-watered plants infested with *T. urticae.* Those infested with *T. evansi* had higher IAA concentrations in normal than deficit-watered and opposite to plants infested with *T. urticae*. The IAA is the central regulator of auxin signalling pathway an auxin response transcription factor (Lackman et al 2011), which are important hormones in plant growth and development. Auxin act positively to stomata opening, but in high concentrations, can inhibit the process (Daszkowska-Golec and Szarejko 2013) and be antagonist to ABA (Tanaka 2006). Furthermore, IAA negatively regulates JA expression genes enabling molecular interplay between auxin and JA signalling pathways (Pauwels et al. 2010), here we determine a positive correlation of IAA and JA.

Zeatin had high concentrations in deficit-watered plants infested with *T. urticae* compare to those infested with *T. evansi* in which Zeatin concentration decreased compared to control. Differences in this hormone concentration between plots where markedly by *T. urticae* induction in deficit-watered plants, though normal-watered plants had little higher zeatin concentration when infested with *T. evansi*. Zeatin is a cytokinin and are mainly involved in plant growth and development, but also act together with auxins in stomatal opening or as process inhibitor (Daszkowska-Golec and Szarejko 2013) and be antagonist to ABA (Tanaka 2006). Auxins and cytokinins could modulated positively (Dervinis et al. 2010) or negatively (Naseem and Dandekar 2012) JA-mediated responses to herbivores and regulating defences against pathogens (Nguyen et al. 2016). Which could explain the significant positive correlation of Zeatin to SA and PI concentration, but antagonist to ACC.

Defensive phytohormones JA and SA showed opposite profile patterns. JA concentrations were induced by all treated plants and higher in deficit-watered plants. Within this group of plants, those infested with *T. absoluta* plus spider mites had the highest JA concentrations. Between deficit-watered plants, those infested with *T. urticae* and in combination with *T. absoluta* had the highest induced JA concentrations. JA activates direct plant defences as proteinase inhibitors and polyphenol oxidases through lipoxigenases pathway (Meriño-Cabrera et al 2018; Sarmento et al 2011; Scott et al. 2010).

Here, we determine positive significant correlations between JA and LOX, IP and PPO, accordingly to defense cross-talk cascades. Some herbivores as *T. evansi* have the ability of silence JA conjugation in the LOX pathway (Alba et al. 2015; Sarmento et al 2011), while *T. urticae* strain is a JA inducer (Kant et al. 2004; Sarmento et al. 2011) some strains are suppressor of JA conjugation (Alba et al 2015). Which seems had occurred on deficit-watered plants, besides the no-JA induction in plants damaged by *T. absoluta*. However it may also mediated by JA-Ethylene defense response and ABA antagonism (Lackman et al 2011) since JA and ABA had a negative correlation.

The SA profile clearly show a major induction in plant concentration due to water deficit and damage by *T. urticae* feeding, meanwhile normal-watered plants had no differences on SA concentrations despite herbivore damage. The SA showed opposite correlations of JA related to ABA (positive), LOX, PPO (negative) and IP (positive). The SA is mainly associated to pathogen infections (Derkensen et al. 2013; Nguyen et al. 2016) and sucking insects as aphids (Zhang et al. 2015) but also supress JA-mediated defences (Diezel et al. 2009; Caarls et al. 2015) been antagonist (Alba et al 2015).

Non-suppressor strains of generalist *T. urticae* induced JA and SA dependent defences, while specialist *T. evansi* and *T. urticae* suppressor strain did not induce defenses independently of JA-SA antagonism (Alba et al 2015). Furthermore, these spider mites species seems to be sensitive to SA defenses but secrete salivary proteins to reduce negative effects of these defenses (Villarroel et al 2016).

Lipoxygenases are also biochemical compounds that approach plant defense responses (Meriño-Cabrera 2018; Yan et al. 2013) even some herbivores are capable to silence LOX responses (Silva et al. 2017b). The LOX profile showed most of plant treatments with higher activities in normal-watered plants except in those infested with *T. urticae* and those with both spider mites. These deficit-watered treated plants and those infested with all herbivore species had induced LOX activities. While in normal-watered plants, where those treated with mechanical damage and all herbivore species.

In the response to herbivory wound, the ABA can stimulate the lipase that release linoleic acid transforming it in JA through the lipoxygenases pathway (Howe et al. 1996). However, the correlation analysis indicate a possible negative crosstalk of LOX with ABA, SA, Zeatin and Proline, but positive to ACC and may explain the low LOX activity in deficit-watered plants. In drought stressed soybean plants, LOX increased in positive correlation to ACC-Ethylene (Tanaka et al. 2005; Xu and Zou 1993).

Proteinase inhibitors had a more solely response to water deficit. Deficit irrigated no-infested and infested as well as mechanical damaged plants induced PI concentrations to highest levels. However, PI concentrations decreased to initial control plants levels, which indicates that water deficit and infestation by two or more herbivore species supress these protein defense. This, when comparing to normal-watered plants, on which most treatments induced PI concentrations, with exception of plants having *T. evansi* with *T. urticae* or *T. absoluta*. PI had negative correlations with ACC and positive to SA and Zeatin for a probably SA mediated pathway.

Different kind of herbivores with the same or different feeding mechanisms induce different JA-defensive genes that codify PI resulting in different defensive expressions (Godinho et al. 2016; Rodriguez-Sanoa et al. 2010; Stam et al. 2014). The spider mite *T. evansi* can supress or silence IP production in tomato (Sarmento et al. 2011) and in simultaneous infestation with *T. urticae* host tomato plants induced mid-levels of PI concentrations (Oliveira et al 2016). Although *T. evansi* were unable of supress PI in normal-watered plants as exclusive herbivory in plants, seemed that could interfered when shared hosts plants with *T. urticae* and *T. absoluta*.

Water deficit and infestation by *T. evansi* on tomato plants have been already study (Ximénez-Embún et al. 2016). Our results agree with this previous research in serino-proteinases inhibitors profile on which *T. evansi* did not supress PI concentrations and drought control and infested plants had similar concentrations.

Related to *T. urticae* experiments on drought stressed cotton plants (Sadras 1998) did not evaluate PI induction, but it developed equally on stressed than well-watered plants. This probably related to similar defense compounds concentrations in both group of plants, as in our results. However, drought stress may enhance cotton resistance to *T. urticae*. Similarly only one study had mixture drought stress with *T. absoluta* infestation on tomato (Han et al. 2014), without evaluate PI concentrations in plants. Nevertheless, *T. absoluta* develops poorly on drought stressed plants, assuming high defensive compounds concentrations or a less palatable host.

Polyphenol oxidases were more active in normal-watered plants seemingly suppressed by *T. urticae* alone and with *T. evansi* reaching before experiment control plants level. In addition, *T. absoluta* alone and with the spider mite species induced intermediated PPO levels. Those plants infested with all three species showed the highest PPO activity. In deficit-watered plants, *T. evansi* alone and with *T. urticae* apparently suppress PPO activity compared to control and all other treated plants. Therefore, PPO had almost same correlations than LOX sharing the highest correlation between them.

PPO are induced also by JA dependent signalling (Bosch et al. 2014) another class of enzyme related to defensive processes against herbivore insects (Meriño-Cabrera et al. 2018) and pathogens (Wang et al. 2017), widely found in plants and induce a kind of anti-nutritive defence after injury. Like in PI, previous work (Ximénez-Embún et al. 2016) showed *T. evansi* supressing PPO activity in drought stressed plants also accordingly with our results.

Water deficit affected tomato crop production, but herbivore infestations did not influenced it. Tomato plants produced more but smaller and lower weight ripped fruits under water deficit compared to optimal-watered plants. However, fruits of deficit-watered plants had major incidence of small tomato borer caterpillars (*N. elegantalis*). Temperature and drought increases affect tomato yield to produce more, but smaller and less quality fruits (Heuvelink 2005: Silva et al. 2016). Our results points to drought increase *N. elengatalis* impacts on tomato crops, which is already the most devastating invader of tomato (*S. lycopersicum*) in the Americas and Caribbean (Silva et al. 2017a).

This work stand out the effects of water deficit in multi herbivore-tomato interactions. Water deficit and herbivory mediated hormonal cascades crosstalk compared to well-watered plants, resulting in different expressions of defensive enzymatic compounds. The generalist spider mite *T. urticae* had a noticeable influence on biochemical tomato compounds in negative (Proline, ACC, LOX, PPO) as positive (AIA, Zeatin, JA, SA) induction. The specialist tomato herbivores *T. evansi* and *T. absoluta* mark most crosstalk responses when sharing host plants with other herbivores than alone. Water deficit affected tomato yield without influence of herbivory and could turn crops more susceptible to tomato borers.

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TABLES

Table 1.1. Two-way Analyses of Variance tables for tomato phytohormones and Proline concentrations (ng/tissue g) of plants under normal and deficit irrigated environments (plots) and ten different arthropod-infested treatments.

Two-way ANOVA parameters					
Phytohormone	**Source of Variation**	**D.F.**	**M.S**	**F**	**P**
Abscisic Acid	Water regime	1	2269.587	233.511	<0.001
	Infestation	9	154.315	15.877	<0.001
	Interaction	9	46.264	4.760	<0.001
	Residual	60	9.719		
	Total	79	58.961		
ACC-Ethylene	Water regime	1	585190.4	390.342	<0.001
	Infestation	9	71339.758	5.177	<0.001
	Interaction	9	25732.466	1.635	0.126
	Residual	60	15735.895		
	Total	79	30417.647		
Indole-3-Acetic Acid	Water regime	1	0.526	1.787	0.186
	Infestation	9	0.589	15.982	0.05
	Interaction	9	0.832	26.324	0.008
	Residual	60	0.295		
	Total	79	0.392		
Jasmonic Acid	Water regime	1	1532.058	20.404	<0.001
	Infestation	9	354.425	4.720	<0.001
	Interaction	9	236.918	3.155	0.004
	Residual	60	75.087		
	Total	79	143.790		
Methyl Jasmonate	Water regime	1	58.858	3.598	0.063
	Infestation	9	20.840	1.274	0.270
	Interaction	9	22.178	1.356	0.229
	Residual	60	16.358		
	Total	79	18.070		
Proline	Water regime	1	2984902272	279.931	<0.001
	Infestation	9	473841584	4.444	<0.001
	Interaction	9	197697446	1.854	0.077
	Residual	60	106629941		
	Total	79	535324943		
Salicylic Acid	Water regime	1	617681.826	87.819	<0.001
	Infestation	9	34065.069	4.843	<0.001
	Interaction	9	34128.900	4.852	<0.001
	Residual	60	7033.570		
	Total	79	20929.642		
Zeatin	Water regime	1	9933.325	1.434	0.236
	Infestation	9	25271.707	3.649	0.001
	Interaction	9	24713.651	3.569	0.001
	Residual	60	6924.752		
	Total	79	11079.578		

D.F. = degrees of freedom; M.S = mean squares; F = Fisher value; P = probability value

Table 1.2. Deviance analysis for linear mixed effects models with Poisson distribution for enzymatic activities.

	Source of Variation	DF	Deviance	Residual DF	Residual Deviance	Pr (> Chi)
Lipoxygenases	Water regime	1	39.802	98	329.90	2.811x10^{-10}
	Infestation	9	92.952	89	236.95	4.154x10^{-16}
	Interaction	9	75.560	80	161.39	1.225x10^{-12}
	Total			99	369.70	
Protease Inhibitors	Water regime	1	31.685	98	342.54	8.879x10^{-5}
	Infestation	9	93.662	89	248.88	5.651x10^{-6}
	Interaction	9	101.581	80	147.30	1.833x10^{-6}
	Total			99	374.23	
Polyphenol oxidases	Water regime	1	8526.6	98	28984	< 2.2x10^{-16}
	Infestation	9	10744.8	89	18239	< 2.2x10^{-16}
	Interaction	9	6060.2	80	12179	< 2.2x10^{-16}
	Total			99	37511	

DF= degrees of freedom; Pr (>Chi) = Probability value higher than Chi square value

Table 1.3. Correlation table of all tomato biochemical variables comprising the overall plant response. Indicating the Spearman correlation coefficient and the p-value (n = 20).

	PPO	PI	ABA	JA	SA	IAA	ACC	Zeatin	Proline
LOX	0.892** < 0.001	-0.414 0.068	-0.685** < 0.001	0.527* 0.016	-0.559* 0.010	0.530* 0.016	0.672* 0.001	-0.475* 0.033	-0.781** < 0.001
PPO		-0.349 0.129	-0.604** 0.005	0.493* 0.026	-0.436* 0.053	0.329 0.153	0.416 0.066	-0.281 0.225	-0.723** < 0.001
PI			0.202 0.388	0.0256 0.911	0.463* 0.039	-0.070 0.762	-0.513* 0.021	0.778** < 0.001	0.408 0.073
ABA				-0.454* 0.044	0.511* 0.021	-0.192 0.410	-0.407 0.073	0.239 0.305	0.691** < 0.001
JA					0.138 0.555	0.598* 0.005	0.213 0.360	0.0895 0.700	-0.621** 0.003
SA						-0.239 0.305	-0.672** 0.001	0.562** 0.009	0.485* 0.029
IAA							0.637** 0.003	-0.231 0.320	-0.407 0.073
ACC								-0.631** 0.003	-0.434 0.055
Zeatin									0.196 0.399

*significance at 95%; ** significance at 99%

ABA = Abscisic acid; ACC = Aminocyclopropane-1-Carboxylic acid; IAA = Indole-acetic acid; JA = Jasmonate acid; LOX = Lipoxygenases; PI = Proteinase Inhibitors; PPO = Polyphenol oxidases; SA = Salicylic acid

Table 1.4. Tomato fruit evaluation by Analysis of Variance (One-way ANOVA) comparing between normal-watered and deficit-watered plants.

Fruit variable	**Water input**	**Mean**	**SEM**	**RDF.**	**RSM.**	**F test**	**P-value**
Quantity	Normal	15.140	0.725	98	30.445	35.769	<0.001
	Deficit	21.740	0.832				
Size	Normal	8.155	0.0752	98	0.300	45.633	<0.001
	Deficit	7.416	0.0796				
Weight	Normal	206.085	7.374	98	1833.432	39.120	<0.001
	Deficit	152.522	4.355				
Bored	Normal	4.440	0.371	98	12.580	10.150	0.002
	Deficit	6.700	0.605				

SEM = standard error mean; RDF = residual degrees of freedom; RSM = residual square mean; F = Fisher; P = probability

FIGURES

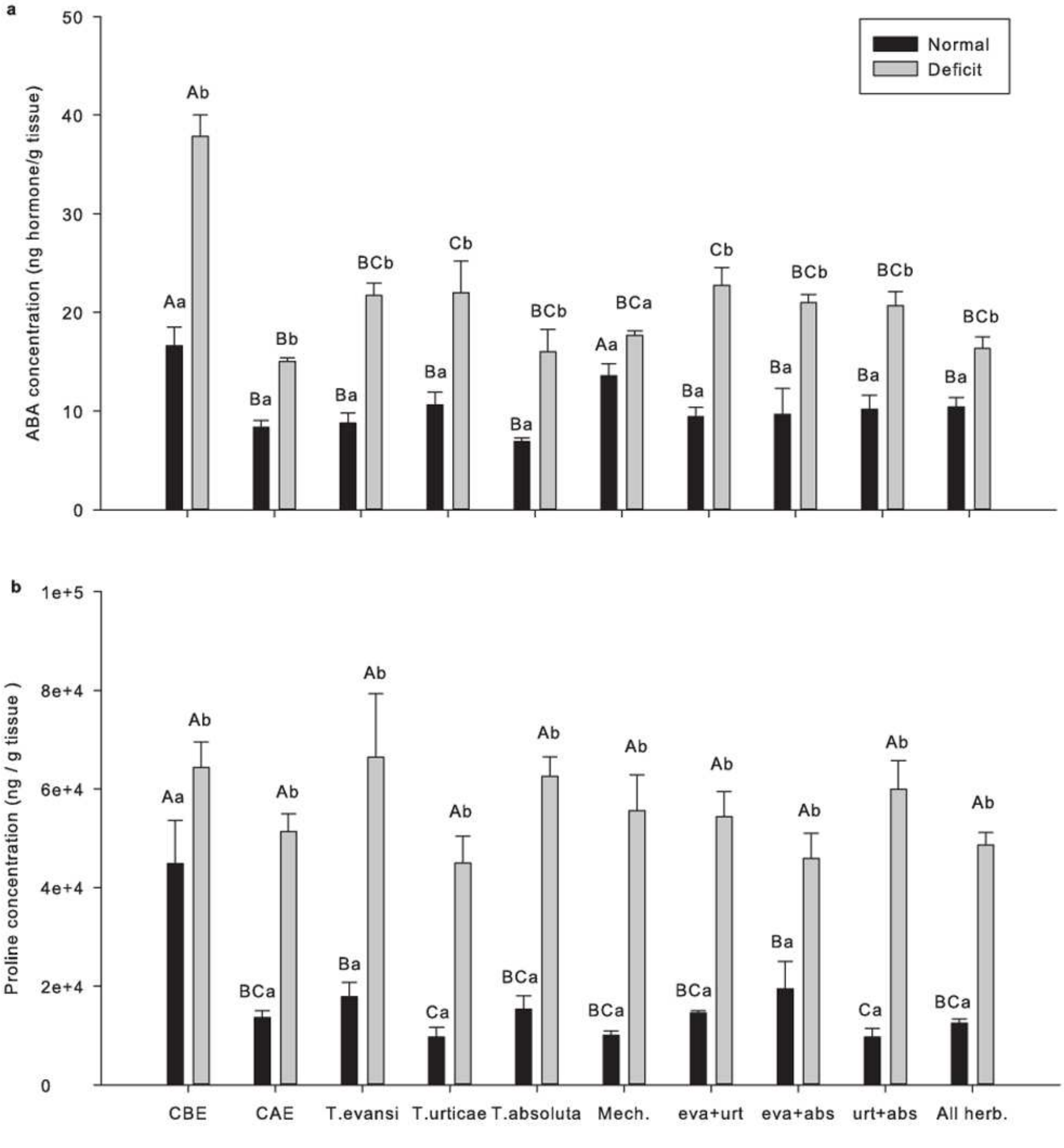

Figure 1.1 Concentrations of physiological phytorhomone ABA (a) and amino acid Proline (b) of tomato plants under deficit and normal water regimes. Control treatments without infestation before (CBE) and after (CAE) seven days of experiment. Treatments with infestation for seven days with; *Tetranychus evansi; Tetranychus urticae; Tuta absoluta*; mechanical damage; *T. evansi* plus *T. urticae*; *T. evansi* plus *T. absoluta*; *T. urticae* plus *T. absoluta*; and all herbivores together. Bars (Mean ± SEM; n = 4) with same lowercase letters indicate no significant difference between irrigation environments and those followed by same capital letters no significant difference between treatments into plots by Student-Newman-Keuls Method at 95% of confidence level.

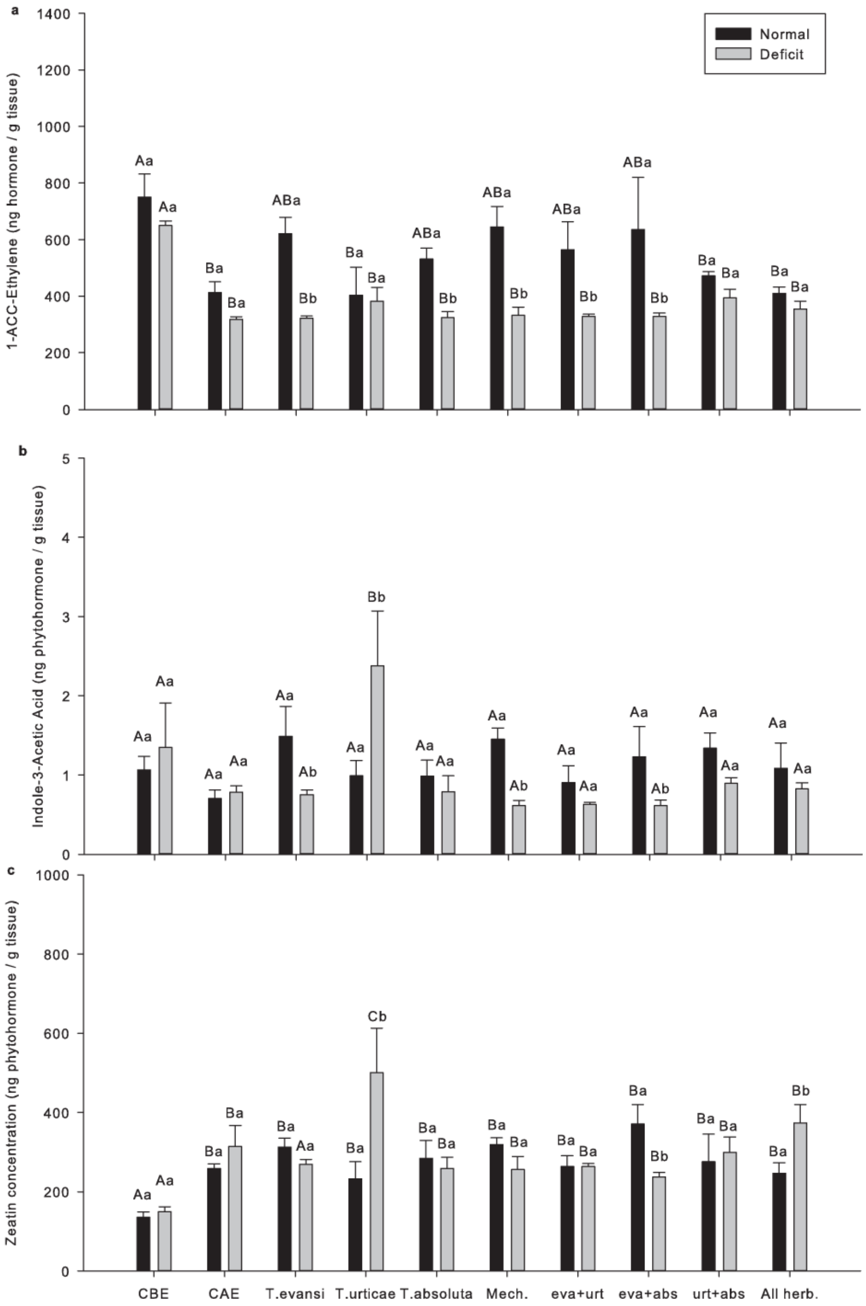

Figure 1.2. Concentrations of physiological phytorhomones ACC-Ethylene (a) IAA (b) and (c) Zeatin of tomato plants under deficit and normal water regimes. Control treatments without infestation before (CBE) and after (CAE) seven days of experiment. Treatments with infestation for seven days with; *Tetranychus evansi; Tetranychus urticae; Tuta absoluta*; mechanical damage; *T. evansi* plus *T. urticae*; *T. evansi* plus *T. absoluta*; *T. urticae* plus *T. absoluta*; and all herbivores together. Bars (Mean ± SEM; n = 4) with same lowercase letters indicate no significant difference between irrigation environments and those followed by same capital letters no significant difference between treatments into plots by Student-Newman-Keuls Method at 95% of confidence level.

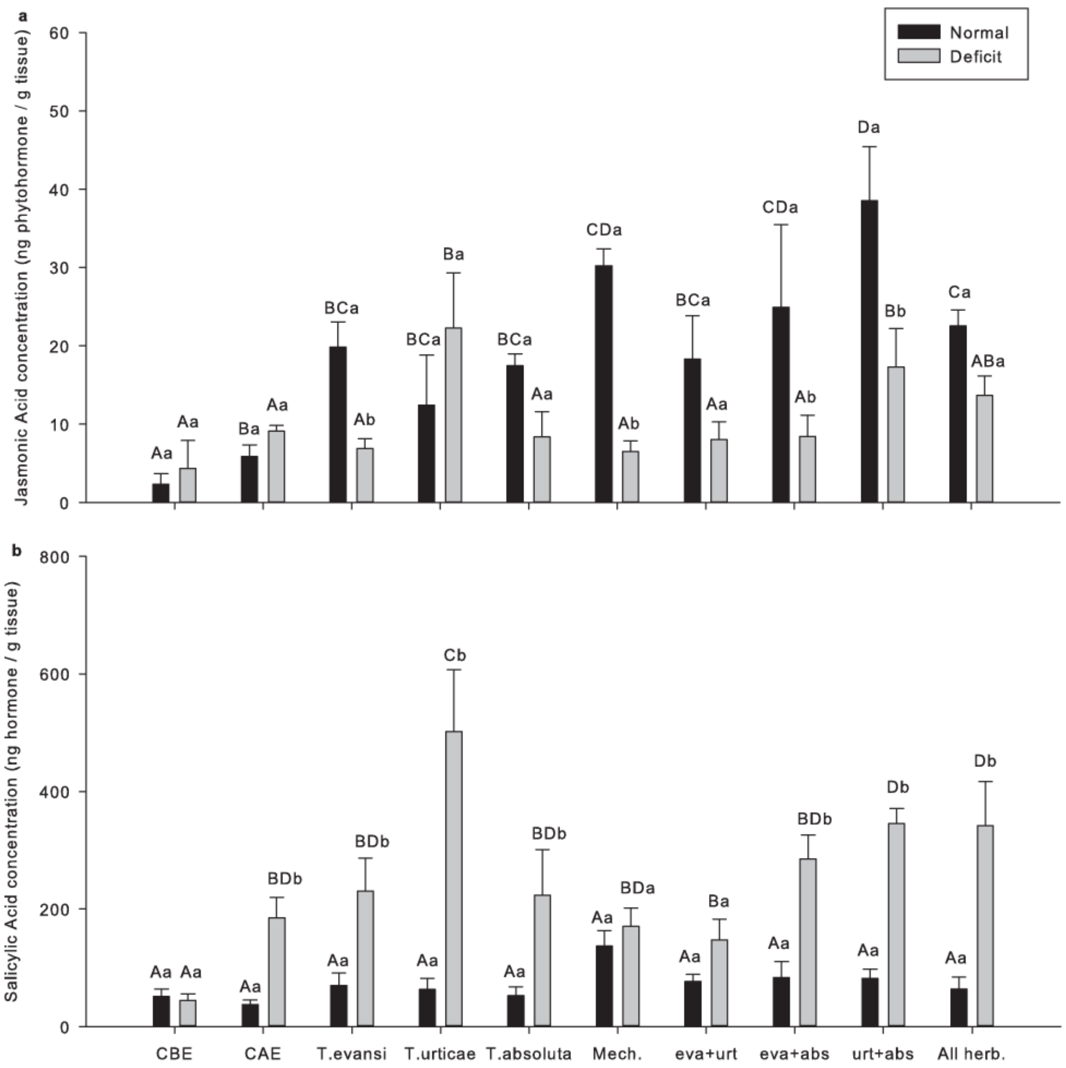

Figure 1.3. Concentrations of physiological phytorhomones Jasmonic Acid (a) and Salicylic Acid (b) of tomato plants under deficit and normal water regimes. Control treatments without infestation before (CBE) and after (CAE) seven days of experiment. Treatments with infestation for seven days with; *Tetranychus evansi; Tetranychus urticae; Tuta absoluta*; mechanical damage; *T. evansi* plus *T. urticae*; *T. evansi* plus *T. absoluta*; *T. urticae* plus *T. absoluta*; and all herbivores together. Bars (Mean ± SEM; n = 4) with same lowercase letters indicate no significant difference between irrigation environments and those followed by same capital letters no significant difference between treatments into plots by Student-Newman-Keuls Method at 95% of confidence level.

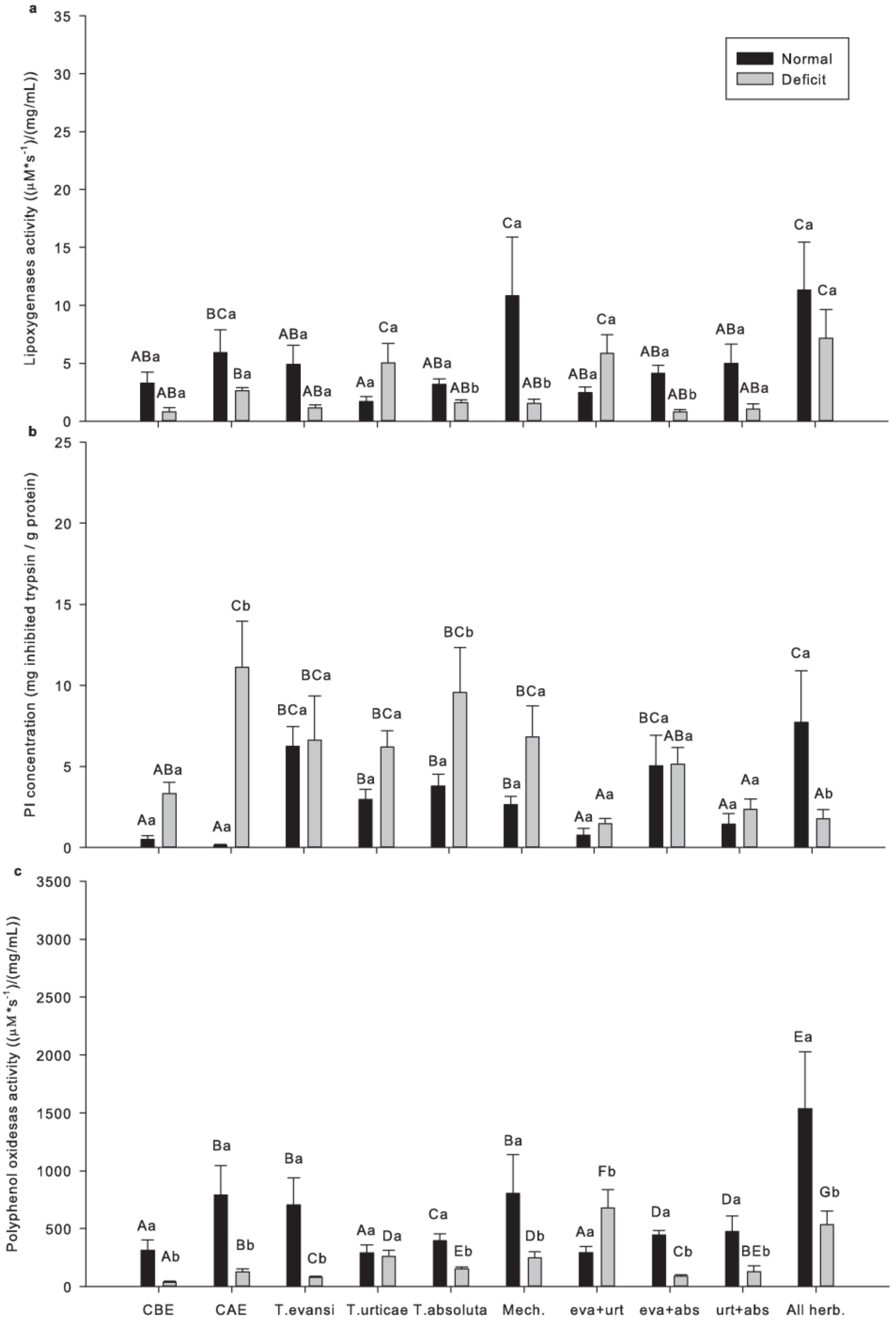

Figure 1.4. Lipoxygenases activity (a) Proteinase Inhibitors concentration (b) and Polyphenol oxidases activity (c) of tomato plants under deficit and normal water regimes. Control treatments without infestation before (CBE) and after (CAE) seven days of experiment. Treatments with infestation for seven days with; *Tetranychus evansi; Tetranychus urticae; Tuta absoluta*; mechanical damage; *T. evansi* plus *T. urticae*; *T. evansi* plus *T. absoluta*; *T. urticae* plus *T. absoluta*; and all herbivores together. Bars (Mean ± SEM; n = 5) with same lowercase letters indicate no significant difference between irrigation environments and those followed by same capital letters no significant difference between treatments into plots by the general linear hypothesis testing 95% of confidence level.

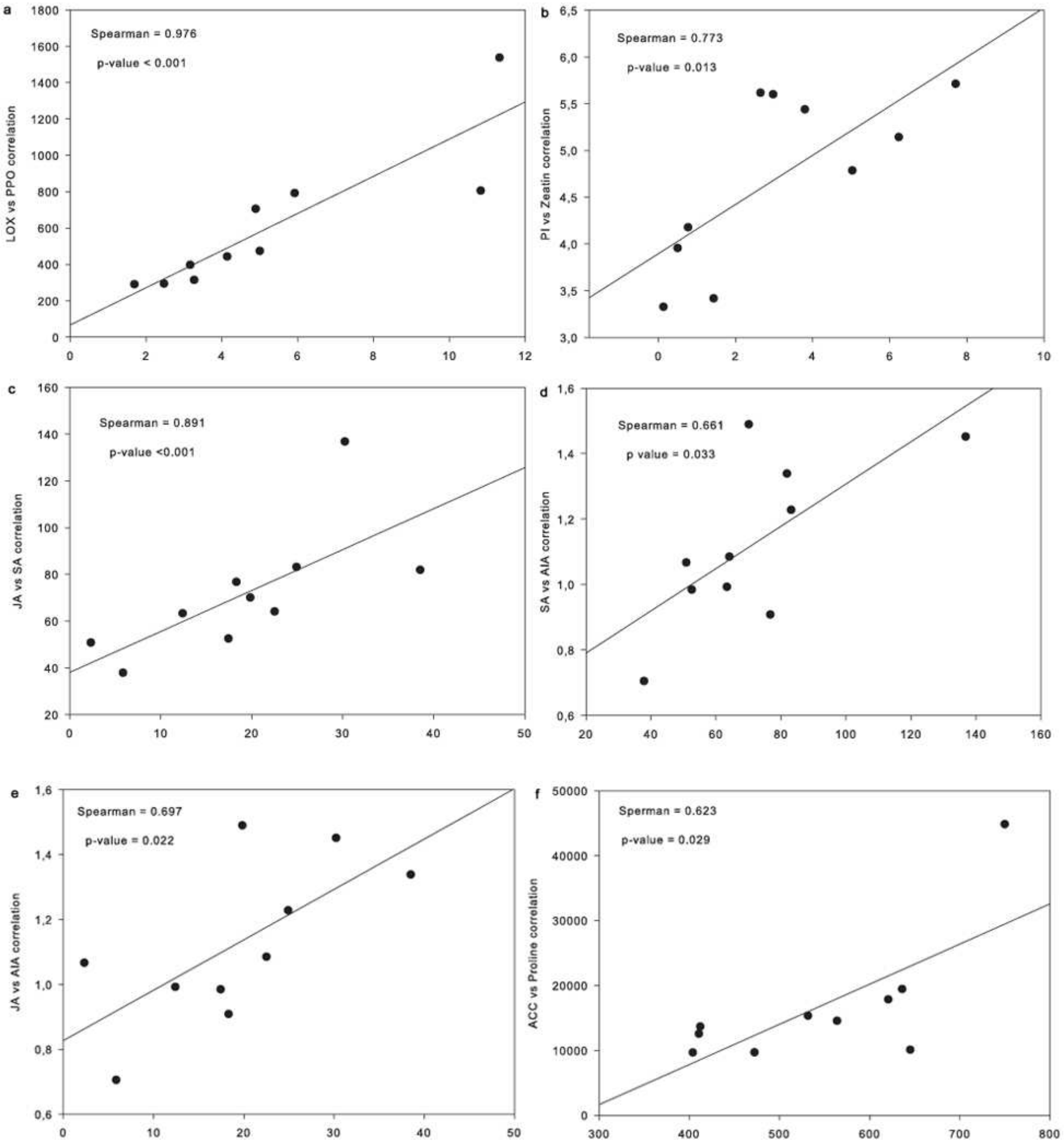

Figure 1.5. Correlation regressions for tomato biochemical compounds of plants under normal-watered regime. (a) Lipoxygenases correlation with polyphenol oxidases; (b) Proteinase inhibitors correlation with Zeatin; (c) Jasmonic acid correlation with Salicylic acid; (d) Salicylic acid correlation with Indole-3-Acetic acid; (e) Jasmonic acid correlation with Indole-3-Acetic acid; (f) 1-Aminocyclopropane-1-Carboxylic acid correlation with Proline amino acid.

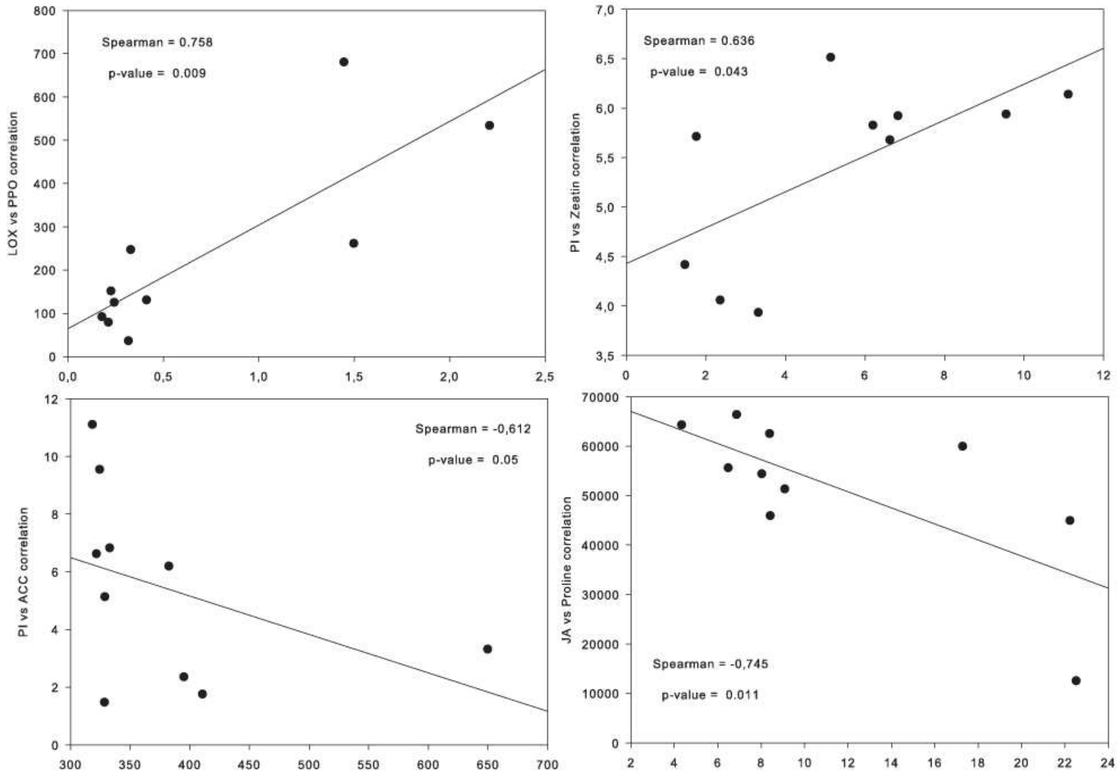

Figure 1.6. Correlation regressions for tomato biochemical compounds of plants under deficit-watered regime. (a) Lipoxygenases correlation with polyphenol oxidases; (b) Proteinase inhibitors correlation with Zeatin; (c) Proteinase inhibitors correlation with 1-Aminocyclopropane-1-Carboxylic acid; (d) Jasmonate acid correlation with Proline amino acid.

CAPÍTULO II: Hydric deficit and herbivory mediate biochemical defenses and resistance of tomato against *Tuta absoluta* (Gelichiidae: Lepidoptera)

Abstract

Water is an important abiotic factor that mediate plant-pest interactions, so water deficit can influence plant chemical defence enhancing or reducing resistance to herbivores. *Tuta absoluta* (Gelechiidae: Lepiodoptera) is one of the most important tomato pests worldwide having an intimate relationship with their host plant. Thus, suitability of host for *T. absoluta* migth be increase due drought forecast by climate change. This work aim to determine effects of water deficit and herbivory by *T. absoluta* under field conditions in the tomato signalling defense transducing and implications on the herbivore performance. So we ask if a combination of water deficit with herbivory can affect the expressions of plant hormones and defense compounds could increase or decrease?; also, if the water deficit affects the development and reproductive success of the tomato moth, *Tuta* absoluta. Tomato crop experimental plot with normal and deficit water input environments were stablished. Six plants from each plot were exposed to *T. absoluta* infestation through adults' ovipositation. Firstly, ovipositation rates as well as of first generation adults were measured. *T. absoluta* survival were also compared. Plant phytohormones and amino acids concentrations, lipoxygenases activity and concentrations of proteinases inhibitors were determined. Initial oviposition of *T. absoluta* adults tend to be higher on plants subjected to water deficit. Survival of caterpillars and pupae were significantly higher also in deficit-watered plants. Abscisic acid and Proline showed higher concentrations on deficit irrigated plants and only Proline response to *T. absoluta* damage. Jasmonic acid were induced by *T. absoluta* only under optimal-watered plants and methyl-jasmonate were higher in no-infested plants in both environments. Salicylic acid shows same profile of abscicic acid and ethylene showing higher concentrations on normal-watered plants, especially those damaged by *T. absoluta*. Lipoxygenases activities had an opposite profile to abscisic and salicylic acids, with major activities on optimal-watered plants, seeming to be supressed by *T. absoluta* damage. Proteinase inhibitors were much higher in deficit-watered plants and were induced by *T. absoluta* damage only on normal-watered plants. Tomato plants under water deficit were more suitable for *T. absoluta* performance despite the herbivore wound induced jamonic acid mediated defences with high proteinase inhibitors production, matching with the “plant stress hypothesis”. Cross-talk signalling of plants phytohormones show noticeable differences between different water inputs and *T. absoluta* herbivory. Evidentially, jasmonic acid/ethylene mediated defensive responses were inhibited by abscisic/salicylic acids antagonism under water deficit.

Keywords; Irrigation, Lipoxigenases, Oviposition; Proteinase Inhibitors, Phytohormones, Survival.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one the most important vegetable crops worldwide (Heuvelink 2005; FAOSTAT 2018), which is subject to the attack of a large number of pathogens and arthropods, practically throughout its life-cycle, from germination to harvesting. *Tuta absoluta* [(Meyrick, 1917) (Lepidoptera: Gelechiidae)] is one of the most important tomato pests, mainly in South America, Europe, Africa and Asia, causing damage to leaves, fruits, flowers and stems. It produces mines and galleries that reduce photosynthetic capacity and severely degrades the crop production (Desneux et al. 2010; 2011).

Damage by herbivores triggers biochemical events resulting in the expression of plant defense genes (Maffei et al. 2007; Sarmento et al. 2011; Scalschi et al. 2013). For example, in the metabolic pathway of lipoxygenases the production of jasmonic acid activates genes that express defense proteins such as protease inhibitors (PIs) (Farmer and Ryan 1992; Scott et al. 2010; Shivaji et al. 2010; Silva et al. 2017). PIs and other compounds, such as tannins and phenols, may affect the ability of herbivores to digest proteins (Meriño-Cabrera et al. 2018; Patarroyo-Vargas et al. 2017). PIs decreases the availability of amino acids and the synthesis of proteins for herbivores growth, development and reproduction (Pilon et al. 2006; 2016; Scott et al. 2010).

Given this, there are many aspects that must influence an herbivore when choosing the best host plant and the correct place to lay eggs. Different sensory cues provide information about the feasibility and quality of resources and the process of this information is a crucial aspect of decision-making (Riffel et al. 2013). For example, aspects such as copulation and feeding during the larval phase modulate the preference in the choice of host plants in *Spodoptera littoralis* (Boisduval, 1833) (Noctuidae; Leidoptera) (Proffitt et al. 2015).

In addition to the biotic factors mentioned above, abiotic factors such as water availability and temperature are important factors that can affect both the productivity and reproductive success of organisms and the interactions between them (Gutbrodt et al. 2011; Inbar et al. 2001; Valim et al. 2016). Various anthropogenic and industrial activities have contributed to an increase in concentrations of greenhouse gases in the atmosphere. This situation has changed the global climate and raised the temperature of the Earth's surface by 0.74ºC in the last century (IPCC 2012).

Climate change, also referred to as global warming, interferes with biological impacts related to the ability of terrestrial organisms, populations, communities and ecosystems to cope with changes so that they can scale up their responses from the molecular to the biosphere level (Peñuelas et al. 2013). Through modelling studies along with statistical projections, long-term predictions of the scenarios affected by climate change impacts indicate changes in crop yields and distribution of insect pests (Elith and Leathwick 2009; Maguire et al. 2015; Silva et al. 2016a;b). These are more vulnerable to climate change and will suffer primarily from the high rate of evapotranspiration due to the increase in atmospheric temperature and the lack of available water in the soil. (White 1969).

The effects of climate change, have been shown in several biological study areas (Shabani et al. 2012; Silva et al. 2016a). Due to its increasing importance, many types of research have been directed to evaluate effects of temperature in lab, but none of them have been undertaken to assess the effect of droughts in biochemical defense response and insect pest development under field conditions. Thus, we aim to determine effects of water deficit and herbivory by *T. absoluta* under field conditions in the tomato signalling defense transducing and implications on the herbivore performance. We ask if a combination od water deficit with herbivory can affect the expressions of plant hormones and if defense compounds can increase or decrease?; also if the water deficit affects the development and reproductive success of the tomato moth, *Tuta absoluta*.

MATERIAL AND METHODS

Tomato crop

Tomato (*Solanum lycopersicum* L.) of Thaíse cultivar from Feltrin seeds (Rio Grande do Sul, Brazil) was used. This cultivar presents tolerance and resistance to tomato mosaic virus, tomato yellow leaf curl virus, *Fosarium oxysporum* Schltdl.

(Nectriaceae; Sordariomycetes), *Verticillium dahliae* Kleb (Plectosphaerellaceae; Sordariomycetes) *Meloidogyne arenaria* (Chitwood, 1949), and *M. incognita* (Kofoid & White, 1919) (Heteroderidae; Secernenta). Seeds were planted in plastic trays (8 x 16 cells) with commercial substrate, covered with plastic film and maintained into a germination chamber at 30ºC for three days. After germination, trays were left in a greenhouse with automatic irrigation system. A protective fertigation were applied twice until transplantation.

After 30 days, seedlings were transplanted to ground where soil was previously treated and fertilized in the experimental plots of the Plant Science Department of the Federal University of Viçosa (UFV), Minas Gerais, Brazil. An irrigation system by drip hosepipe and a tutoring vertical system were installed (supplemental material; SM1). Tomato crop consisted in two plots of six lines containing 14 plants spaced 0.6 m between them and 1 m between lines. Normal irrigation were maintained for two weeks before experimentation and controlled irrigation were then applied to both plots. One of them with normal water supplied considered as 100% irrigation for ideal crop management and production, it means a proportion of 8L/m^2/day and 3L/plant/day (Heuvelink 2005; Silva and Vale 2007). In the other plot were applied a restricted irrigation from 50% to 25% without compromising the plants development.

A protective spraying (supplemental material; SM5) were applied twice per week for one and a half month since transplantation and then stopped before experimental treatments be performed. Fertigation, weeding and stripping crop management were executed for ideal production using local procedures based on standard agronomic practices (Alvarenga 2013). The infestation experiment was performed after pesticides effect ceased and the irrigation treatment of crop was stabilized to 100% and 50% irrigation.

Meteorological measurements from a field station (IrriPlus E5000) were downloaded and inserted in an Excel (Microsoft Office ®) spreadsheet for irrigation time be determined every two to three days. Soil samples at 40 cm deep were taken with a manual auger. Soil humid weight was registered and then dried in a lab's stove for 24 hour at 100 ºC. The dry weight was registered and the differences between plots were compared (supplemental material; SM2). The Real Irrigation Needed (RIN) were determine in a tow to three days frequency by estimating the Crop Evapotranspiration (ETc), using adjustment coefficients over the Reference Evapotranspiration (ETo) of tomato, combined with meteorological, soil, water, plant and equipment data on the Penman-Moteith-FAO56 model (Allen et al. 1998; Delazari et al. 2016; see supplemental material; SM3 for equations).

Tuta absoluta infestation, oviposition, survival and development

Newly emerged moths of *T. absoluta* were obtained from the Integrated Pest Management laboratory of the Entomology Department at UFV. Twelve groups of eigth to ten moths (50:50 male:female proportion) were caught and placed in plastic recipients and then taken to field experimental crop. Six plants between V4 and V5 vegetative stage from each plot sector were randomly selected. The third fully developed leaf from apex to middle plant were covered with an organza bag and the *T. absoluta* adults were placed inside the bags for 48 hours for oviposition (initial oviposition).

After this, the adults were sacrifice and the eggs counted and reported in a tomato leaf design in a bold letter paper. One day latter the eggs were counted again for verification and four days after the small leaf mines also counted and reported in the design. This verification were made every three days for caterpillar determination and survival accounting. Caterpillars were left to pupae inside the organza bags. Then leaves were cut off keeping the bags covering it and taken to laboratory for pupae accessing, weighting and sex determination. Survival were accounted until adults' emergence.

Survival pupae were kept separated by plant plot and sex. Once most adults emerged every group of adults per plant were reunited inside a wood cage covered with organza to mate and egg oviposition (F1 oviposition) over a tomato leaf in laboratory conditions at 27 ± 5 ºC and 70 ± 10 % RH. Eggs were counted every 48 hours until adults' death.

Plant physiological and biochemical defense response

Sample collecting

Leaflets without presence of mines from infested and systemic leaves were selected for biochemical analyses. In addition, no-infested plants from both plots were selected as control. The leaflets were cut with scissors and put inside aluminum foil envelops and immediately bathed with liquid nitrogen inside a Styrofoam box and rapidly toke to a -80 ºC freezer.

Phytohormones and amino acids

For extracts preparation the leaflets were macerated in liquid nitrogen and the tissue weighted. For each 100 mg of tissue 200 µL of the extractive solution (methanol 20% / Isopropanol 79% / Acetic Acid 1%) subjected to ultrasound for 30 min and centrifuged at 14000 *g* for 30 min at 4 °C. The supernatant was collected, and the procedure repeated. The extracts were stored at -80 °C until analysis in the LC-MS of type UHPLC QqQ been performed.

Approximately 300 µL of the extracted samples were filtered and placed in vials, then 5.0 µL of the extracts were injected into the LC / MS system of the Nucleus of Biomolecules Analyzes (NuBioMol) of Helath and Biological Sciences Center of UFV. It was used a chromatographic column (Agilent Eclipse Plus, RRHD, 1.8 um, 2.1x50 mm) with flow of 0.3 mL / min coupled online to a triple quadrupole mass spectrometer QqQ (Agilent). The phytohormones analyzed were Abscisic Acid (ABA), Jasmonic Acid (JA), Methyl Jasmonate (Me-Ja), Salicylic Acid (SA), 1-Aminocyclopropane-1-carboxylic acid (ACC-Ethylene) and the amino acid Proline. The mass spectrometer operated by alternating positive and negative mode according to retention time for each hormone and monitoring the sample in MRM mode (*multiple reaction monitoring*) to allow the hormones detection.

The assays were performed with six biological replicates of each treatment. The mass spectra generated were processed using the MassHunter (Agilent Technologies) software to obtain the chromatograms extracted (XIC) of each transition and to obtain the area values as indicative of the abundance of each hormone. Area values were adjusted using Skyline sortware (MacCoss Lab; University of Washington) and exported to Excel spreadsheet. A standard curve of each hormone, varying concentrations of 0.1 to 300 ng / mL, was used to convert the area values of the identified metabolites into concentration values expressed as (ng of hormone / g of leaf tissue; see SM3 for equation).

Enzymatic activities

The plant material of each plant was weighed in analytical balance, macerated in liquid nitrogen, transferred to sodium phosphate buffer solution 0.05M pH 6.5 (lipoxygenases analysis) or buffer solution Tris-HCl 0.1M, pH 8.2 with 20mM of $CaCl_2$ (proteinase inhibitors analysis); 1:3 (w/v). The extracts were centrifuged at 17200 *g* by

60 minutes at 4°C (Meriño-Cabrera et al. 2018) and the supernatant used for the determination of the enzymatic activities. The protein concentration was determined from the extracts using bovine serum albumin (BSA; 0-0.2mg/mL) (Bradford 1976). Enzymatic activities were determined using a Hitachi Ratio Beam Spectrophotometer U-5100 of the Enzymes, Proteins and Peptides Biochemical Laboratory of the Institute of Biotechnology Applied to Agriculture (BIOAGRO, UFV).

Lipoxygenases

The activity of lipoxygenases on linoleic acid was determined (Axelrod et al. 1981), in a mixture of 10 µL of the leaf extract and 20 µL stock solution of sodium linoleate in 100 µL of sodium phosphate buffer. The absorbance of the reaction mixture was determined every 30 seconds at 234 nm for 150 seconds. The final absorbance values resulting from differences between the absorbance at 150 and 30 seconds. The specific activity of product formation were calculated from the final absorbance values in ($\mu mol.s^{-1}$) / (mg/mL) using the molar extinction coefficient of $25000 M^{-1} cm^{-1}$ (see SM3 for equations).

Proteinase Inhibitor

Proteinase inhibitor concentrations in the foliar extract were determined by inhibition of the comercial trypsin activity acting with the foliar extract on substrate N-α-Benzoil-L-arginine 4-nitroanilide hydrochloride (L-BApNA) 1,2 mM (Erlanger et al. 1961). The control of purified trypsin had 100 µL of trypsin solution 4.7×10^{-5} M in micro tube with 1100 µL of buffer Tris-HCl and 500 µL of subtrate. The samples had a micro tube with 500 µL buffer that received 100 µL of foliar extract and 100 µL of trypsin solution. After five minutes of incubation, this solution was transferred to another micro tube with 500 µL buffer and 500 µL substrate. The absorbance of the test and control solutions was determined at 410 nm every 30 seconds for 150 seconds of reaction. The final absorbance were calculated by the differences between the absorbance at 150 and 30 seconds. The results were converted to mg of trypsin inhibited per gram of protein (Kakade et al. 1974; see SM3 for equation).

Statistical analyses

Tuta absoluta on 100% and 50% irrigation plots were compare by t-Student tests. Caterpillar survival on both environments were determined by the Kaplan-Meyer method and compare by the Log-Rank test to significant level of 1%. The phytohormones concentrations the enzymatic activities were analysed in a balanced design by a Two-way Analyses of Variance with power performed test alpha of 0,05 probability. Treatment means were compare by pairwise multiple comparison Tukey test at 5% significant level.

Caterpillar survival analyses were performed using statistical software R version 3.4.3 (R Core Team 2017) *"survival"* package. The rest of analyses were performed using the SigmaPlot 12 (Systat Sofware, Inc.)

RESULTS

Oviposition rates

Initial oviposition do not show significant differences between environments (d.f = 10; t = 1.172; P = 0.268), however oviposition rate in plant subject to deficit of water tend to be higher with a mean rate of 4.408 eggs per female in a 48 hours period, against a mean rate of 2.875 in plants under normal irrigation conditions (Fig. 2.1a). Females whose larval stage fed on plants subjected to irrigation deficit did not had different oviposition rate than those fed on plants under normal irrigation, neither (d.f. = 14; t = -0.499; P = 0.625) (Fig. 2.1b).

Tuta absoluta survival

Tuta absoluta survival was of 42.3% on plants subjected to ideal irrigation and of 10.4% on plants under deficit irrigation. The 50% survival were reached at last larval instars (25 days after oviposition) for caterpillars fed on normal irrigated plants and at pupae stages (31 days after oviposition) for caterpillars fed on deficit irrigated plants (Fig. 2.2). Survival was significant different between irrigation conditions (d.f. = 1; $X^2 = 61$; $P = 5.77 \times 10^{-15}$). Pupal weight did not show differences between environments (d.f. = 10; t = -0.488; P = 0.636) (SM2).

Phytohormones and amino acids response

Two-way ANOVA analyses for ABA results in only water regimes been significant. For Proline, water regimes and infestations show significant differences but not for their interaction (Table 2.1). Phytohormones associated to water deficit, abscisic acid (ABA) and amino acid Proline had higher concentrations in watered-deficit plants and show significant differences between water regimes but infestations, only for Proline concentrations in plants infested and control under deficit watered (Figure 2.3).

Two-way ANOVA analyses for JA indicates significant differences between water regimes, infestation and their interaction. However, only between infestations for MeJa, only between water regimes for SA and only between water regimes and infestations for ACC-Ethylene (Table 1). Defensive phytohormone jasmonic acid (JA) show higher concentrations on tomato plants under normal than deficit-watered environment and those infested with *T. absoluta* (Fig. 2.4a). Methyl jasmonate (MeJa) show higher concentrations on no-infested plants and were different between environments (Fig. 2.4a). Contrarily to salicylic acid (SA) concentration, which were different between plants on plots without been affected by *Tuta absoluta* damage (Fig. 2.4b). The ACC-Ethylene have similar profile to JA, with higher concentrations under normal-watered plots in infested plants followed by no-infested. ACC-Ethylene did not showed differences between plants under deficit-watered plants.

Enzymatic defense response

Lipoxygenases activity were higher in not infested plants under normal irrigated environment and decreased with *T. absoluta* damage. Same pattern was observed in plants under deficit-watered environment. Lipoxygenase activity of no infested plants in normal irrigated plot were significantly different from plants in deficit irrigated plot. However, plants infested with *T. absoluta* do not differ between plots (Fig. 2.4a). Two-way ANOVA analyses showed significant differences between plots and treatments, but their interaction.

Both infested and no infested plants showed equal high concentrations of proteinase inhibitors under deficit-watered environment. Under normal-watered environment, infested plants showed higher PI concentrations than no infested, indicating induced defence response (Fig. 2.4b). Two-way ANOVA analyses showed significant differences only between plots (Table 2.2).

DISCUSSION

Bottom-up forces shape plant-arthropod interactions (Han et al. 2014), such as plant nutritive content, biochemical defences, competitors, light, temperature, water and nutrients availability (Inbar et al. 2001; Gutbrodt et al. 2011). Hormones and hormonal cross-talk play an important role in plant responses to stresses occurring simultaneously in the environment, such as abiotic stresses and herbivory (Nguyen et al. 2016).Water is an important abiotic factor that mediate plant-pest interactions (Gutbrodt et al. 2011; Han et al. 2014). Thus, drought stress can influence plant chemical defence enhancing or reducing resistance to herbivores (Inbar et al. 2001; Huberty et al. 2011). Stressed plants can be more profitable and better hosts to some insects (White 1969). *Tuta absoluta* is one of the most important tomato pests worldwide (Desneux et al. 2010; 2011) and as a leafminer caterpillar have an intimate relationship with their host plant. Thus, this result is an indicative that suitability of host for *T. absoluta* will be increase due drought forecast by climate change according published models and scenarios for future (IPCC 2012; Silva et al. 2016a;b). In addition, this fact is an ideal subject for studies on water stressed plant-arthropod interactions (Inabar et al. 2001) and useful for plant geneticists who could develop tomato cultivars with tolerance to drought stress and resistance to *T. absoluta*.

Oviposition rates were not significantly affected by irrigation treatments. However, our data indicates a tending oviposition rate to be higher on plants under normal irrigated environment. Thus indicating a possible effect of plant water deficit on *T. absoluta* adults chosen the best host. Non-optimal water input would affect the plant vigour that could lead to an herbivore reject (Price, 1991). Females from caterpillars fed on plants under deficit-watered plants neither showed effects on their egg production compare to females feed on normal-watered plants.

This was observed in pupal weight too, which could be associated to a mid than a high water deficit applied. This has been already observed comparing optimal water supply against low and high drought stress. Where under the first two conditions *T. absoluta* pupae had equal mass gain but higher than pupae under high drought stress, what intensified when combined with excessive or poor nitrogen inputs (Han et al. 2014).

Opposite of expected (Han et al. 2014), survival of *T. absoluta* were significantly higher on deficit-watered plants, than plants under optimal water supply. Although been a low survival rate of 42.3% on deficit-watered plants, it is widely higher against a 10.4% on normal-watered plants. The first group reached the 50% survival in pupal stage and the second at larval stages. This indicating a possible nutrient rich condition for plants in the deficit-watered plot since optimal fertilizations were applied in both experimental plots.

This result also could match with the "plant stress hypothesis" (Mattson et al. 1987) which propose that stressed plants are more profitable host to herbivore insects due to increased nutritional value and reduced plant defensive compounds (White 1984). Besides, the insect guild play an important role in this plant responses and insect performance (Larsson, 1989).

Plant physiological stress were accessed through concentrations of phytohormone ABA and amino acid Proline. Results clearly reflect that plants of the deficit-watered plot respond to this condition by increasing their concentrations. In addition, *T. absoluta* damage increased even more the Proline concentration under water deficit. ABA is a response regulator to abiotic stresses, such as drought (Nguyen et al. 2016), due to its involvement in stomatal movement and leaf senescence (Daszkowska-Golec and Szarejko 2013; Liang et al 2014).

It is also known that ABA is require to respond to herbivore damage in tomato and a deficiency of ABA would increase plant susceptibility (Thaler and Bostock 2004). However, our results indicated that ABA did not respond to *T. absoluta* damage, but it could be acting synergistically with JA to enhance resistant to herbivore (Vos et al. 2013) even if *T. absoluta* had a higher survival rates in water deficited plants.

Proline is a free non-essential amino acid that reflects water deficit stress and at the same time may increase the plant susceptibility to herbivore insects, due to act as a feeding stimulant (Schoonhoven et al. 2005; Meyer et al. 2006; Ximénez-Embún et al. 2016). Thus, according to the "plant stress hypothesis" (Mattson et al. 1987), but also as an osmoprotectant (Suzuki et al. 2014). This may explain why *T. absoluta* had higher survival rates in deficit-watered plants, and pupae did not differ in mass weight between deficit and normal irrigated environments.

Proline were higher induced in plants infested with *T. absoluta* under deficit-watered environment. It means that besides responding to water deficit also respond to the herbivore damage. Opposite to what occurred in also tomato plants infested with herbivorous red spidermite *Tetranychus evansi* under drought stress (Ximénez-Embún et al. 2016).

Jasmonic acid concentration were higher on infested plants under normal-watered environment than deficit-watered and both non-infested treatments. *Tuta absoluta* induce JA only on normal watered plants and did not response to water deficit. This, corresponding over again to survival rates of *T. absoluta*, given that JA activates direct plant defences as proteinase inhibitors and polyphenol oxidases (Farmer and Ryan, 1992; Howe et al. 1996; Scott et al. 2010), those were higher in plants were *T. absoluta* had lower survival rates. Also firming up the “Plant stress hypothesis”

Hormone cascades act in no-additive ways enhancing plant tolerance and resistance to some types of stress, but others (Foyer et al. 2016; Stam et al. 2014). The hormone ABA can activate Jasmonate-dependent defences, even systematically (Vos et al. 2013), but also Ethylene hormone (ET) mediates some JA-responsive defenses and this is antagonist to ABA, which negatively affects some JA/ET- dependent defenses, such as nicotine biosynthesis in tobacco plants (Lackman et al. 2011). This antagonist interaction could explain why JA were not induced in deficit-watered plants, since ABA were highly induced.

The Methyl-Jasmonate profile showed to be higher on no-infested plants than infested and were slightly different in plants under deficit-watered environment. This hormone were not induced by *T. absoluta* damage, unlikely it seems that it was supressed through the signalling pathway, maybe by ABA/ET antagonism as anteriorly exposed. Therefore it response to water deficit only in not herbivore damaged plants, so its function to abiotic stress might were supressed too (Wasternak and Parthier 1997).

It is important to have in mind that jasmonates are important compounds in direct defences against herbivores and pathogens, inclusive in indirect defences acting as alarm volatiles as MeJa and protection against abiotic stress. Nevertheless, jasmonates also play an extremely important role as cellular regulators in other physiological plant functions as seed germination, root growth, fertility, fruit ripening and senescence (Cheong and Choi 2003).

Plants under deficit-watered environment had higher concentrations of SA, but without be induced by *T. absoluta* damage. This profile were very different to jasmonates and similar to ABA. In spite of SA been mainly associated to pathogens infections (Derkensen et al. 2013; Nguyen et al. 2016) and sucking insects as aphids (Zhang et al. 2015) but also supress JA-mediated defences (Diezel et al. 2009; Caarls et al. 2015). It seems that SA is responding to water deficit and under normal-watered environment SA were silenced and Jasmonates were highly induced. Thus, *T. absoluta* damage trigger a negative cross-talk between JA and SA (Kawazu et al. 2012; Thaler et al. 2002) on normal-watered plants which is also mediated by ABA when water deficit is present. It is also know that SA acts synergistically with ABA to stomatal closure under drought stress in *Arabidopsis* plants (Miura et al. 2012), so it could explain the relation of similar profiles on both ABA and SA.

The ET show equal concentrations on plants under deficit-watered environment without acting to *T. absoluta* damage. However, it were highly induced by this herbivore in optimal watered plants, responding similar to JA. Thus, ET might be mediating JA defences, given that it acts more like a modulator than an elicitor of herbivore induced responses (Nguyen et al. 2016; von Dahl and Baldwin 2007). ET is also known as a major inducer of leaf senescence (Kim et al. 2015) and an antagonist to ABA in stomatal movement in *Arabidopsis* plants under drought (Tanaka et al. 2015).

Lipoxygenases responded contrary to expected, with major activity on plants in normal-watered environment and without *T. absoluta* damage. In spite of damage cause by this herbivore lipoxygenases activity seems to have supressed in both environment conditions. When an insect wound the plant tissue, it is transduced by a lipase, which facilitates the release of linoleic acid from membrane lipids and stimulated by ABA. Then the linoleic acid is converted to JA through the octadecanoid pathway of lipoxygenases (Farmer and Ryan 1992, Howe et al. 1996). Lipoxygenases inhibitors, ET inhibitors and SA can block this conversion and eliminate PI induction by decreasing the JA synthesis (Koiwa et al. 1997). The lipoxygenases activity profile is related to phytohormones concentration could be explain by the signal transduction cross-talk anteriorly exposed.

Opposite to the lipoxygenases activity, concentration of proteinase inhibitors were highly induced by water deficit and only by *T. absoluta* in normal-watered environment. Tomato plants were unable of produce more PI on deficit-watered plants to respond to the herbivore damage. Thus, putting all defensive capacity for the drought condition but without response to insect wound that could progressively took to a PI diminution, thus making the plant more palatable to *T. absoluta* or the capability of the herbivore to circumvent PI toxic effects (Agrawal and Weber 2015; Meriño-Cabrera et al. 2018; Silva et al. 2017)

Tomato plants under water deficit were less suitable for *T. absoluta* performance due the herbivore wound induced JA mediated defences with high PI production, contradicting with the “plant stress hypothesis”. Cross-talk signalling of plants phytohormones show noticeable differences between different water inputs and *T.*

absoluta herbivory. Evidentially, JA/ET mediated defensive responses were inhibited by ABA/SA antagonism under water deficit.

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TABLES

Table 2.1. Two-way Analyses of Variance phytohormones of tomato plants under deficit or normal watered conditions and infested or not with *Tuta absoluta* (Meyrick, 1917) (Gelechiidae: Lepidoptera).

Two-way ANOVA parameters					
Phytohormone	**Source of Variation**	**D.F.**	**M.S**	**F**	**P**
Abscisic Acid	Water condition	1	338.4	88.01	<0.001
	Herbivory	1	1.314	0.342	0.565
	Interaction	1	5.072	1.319	0.264
	Residual	20	3.845		
	Total	23	18.33		
Proline	Water condition	1	$1.06x10^{+5}$	390.342	<0.001
	Herbivory	1	$13.9x10^{+7}$	5.177	0.034
	Interaction	1	$8.06x10^{+7}$	2.985	0.099
	Residual	20	$2.7x10^{+7}$		
	Total	23	$49.1x10^{+7}$		
Jasmonic Acid	Water condition	1	64.604	6.328	0.021
	Herbivory	1	163.151	15.982	<0.001
	Interaction	1	268.726	26.324	<0.001
	Residual	20	10.208		
	Total	23	30.463		
Methyl Jasmonate	Water condition	1	4.573	3.058	0.096
	Herbivory	1	39.339	26.306	<0.001
	Interaction	1	3.759	2.513	0.129
	Residual	20	1.495		
	Total	23	3.373		
Salicylic Acid	Water condition	1	141849.16	24.985	<0.001
	Herbivory	1	4539.6	0.800	0.382
	Interaction	1	760.301	0.134	0.718
	Residual	20	113547.39		
	Total	23	11334.628		
ACC-Ethylene	Water condition	1	103852.57	38.605	<0.001
	Herbivory	1	25241.982	9.383	0.006
	Interaction	1	18140.825	6.744	0.017
	Residual	20	2690.115		
	Total	23	8740.769		

DF = degrees of freedom; MS = mean square; F = Fisher value; P = probability value

Table 2.2. Two-way Analyses of Variance lipoxygenases activity and proteinase inhibitors concentration of tomato plants under deficit and normal watered conditions and infested or not with *Tuta absoluta* (Meyrick, 1917) (Gelechiidae: Lepidoptera).

	Two-way ANOVA parameters				
	Source of Variation	**D.F.**	**M.S**	**F**	**P**
Lipoxygenases	Water condition	1	35.431	8.197	0.010
	Herbivory	1	21.484	4.970	0.037
	Interaction	1	4.445	1.028	0.323
	Residual	20	4.322		
	Total	23	6.426		
Proteinase Inhibitors	Water condition	1	229.502	5.588	0.028
	Herbivory	1	62.800	1.529	0.231
	Interaction	1	137.946	3.358	0.082
	Residual	20	41.074		
	Total	23	54.423		

DF = degrees of freedom; MS = mean square; F = Fisher value; P = probability value

FIGURES

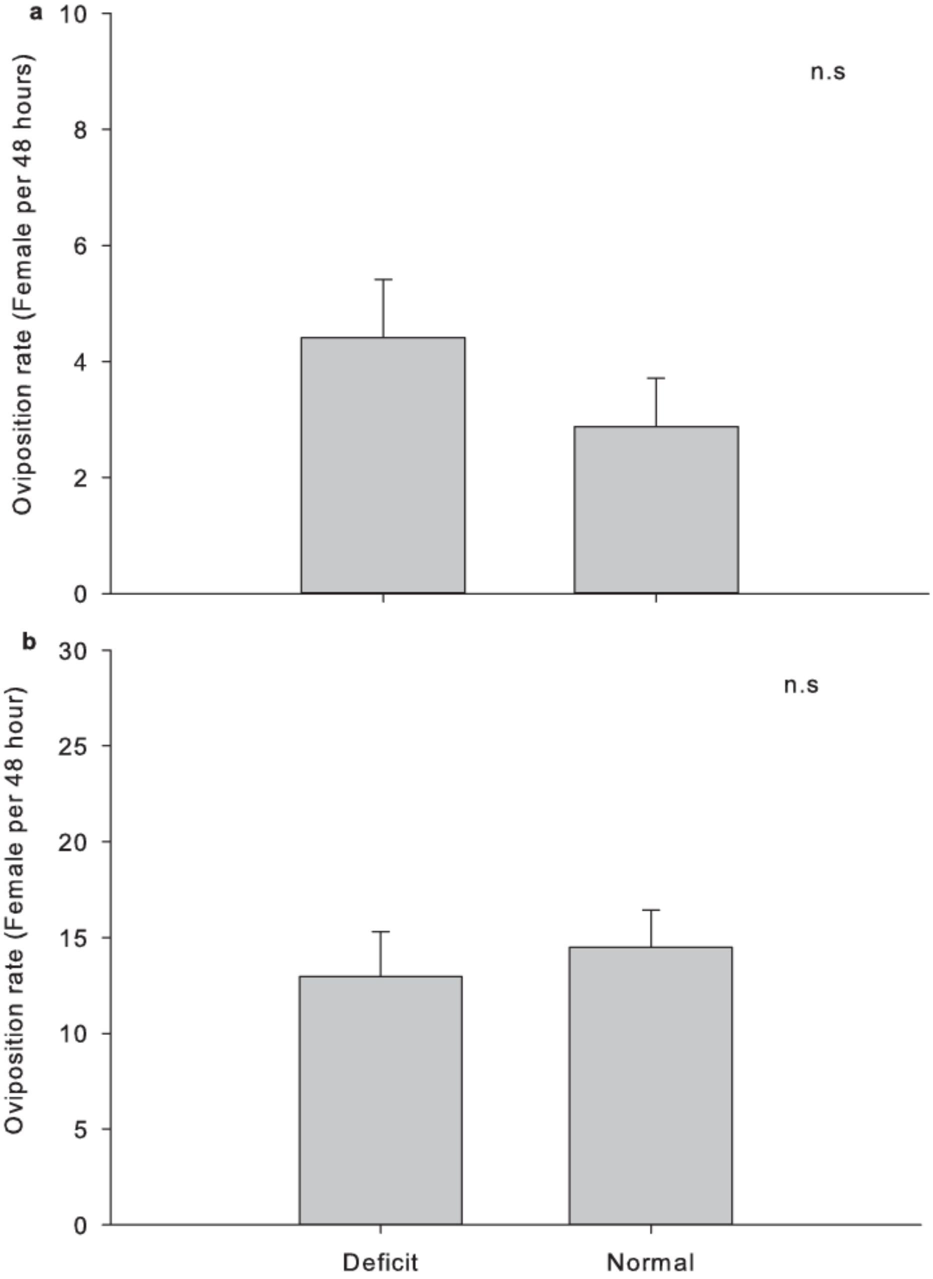


Figure 2.1. Oviposition rates of *Tuta absoluta* (Meyrick, 1917) (Gelechiidae: Lepidoptera) in tomato plants subjected to water deficit and normal condition; (a) Oviposition rates on infested tomato plants at field experimental plots; (b) Oviposition rates of females developed on tomato plants (F1). Pairwise mean comparisons do not differ by t-Student test at 95% of confidence level.

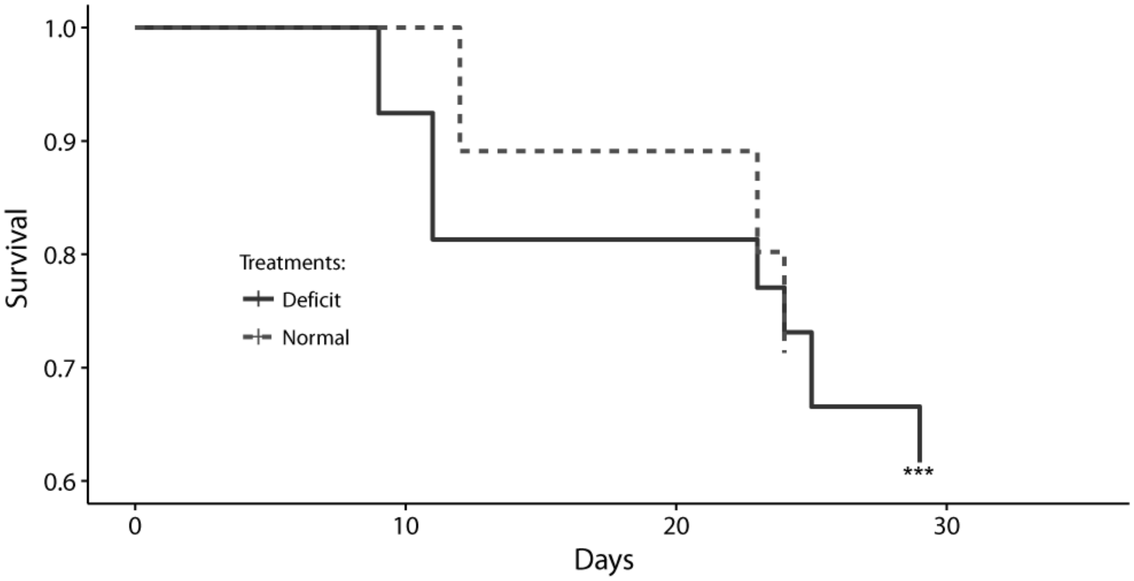


Figure 2.2. Kaplan-Meyer method for *Tuta absoluta* (Meyrick, 1917) (Gelechiidae: Lepidoptera) immatures survival fed on tomato plants subjected to deficit and normal irrigation. Treatments were different by Log-Rank test at 1% significant level.

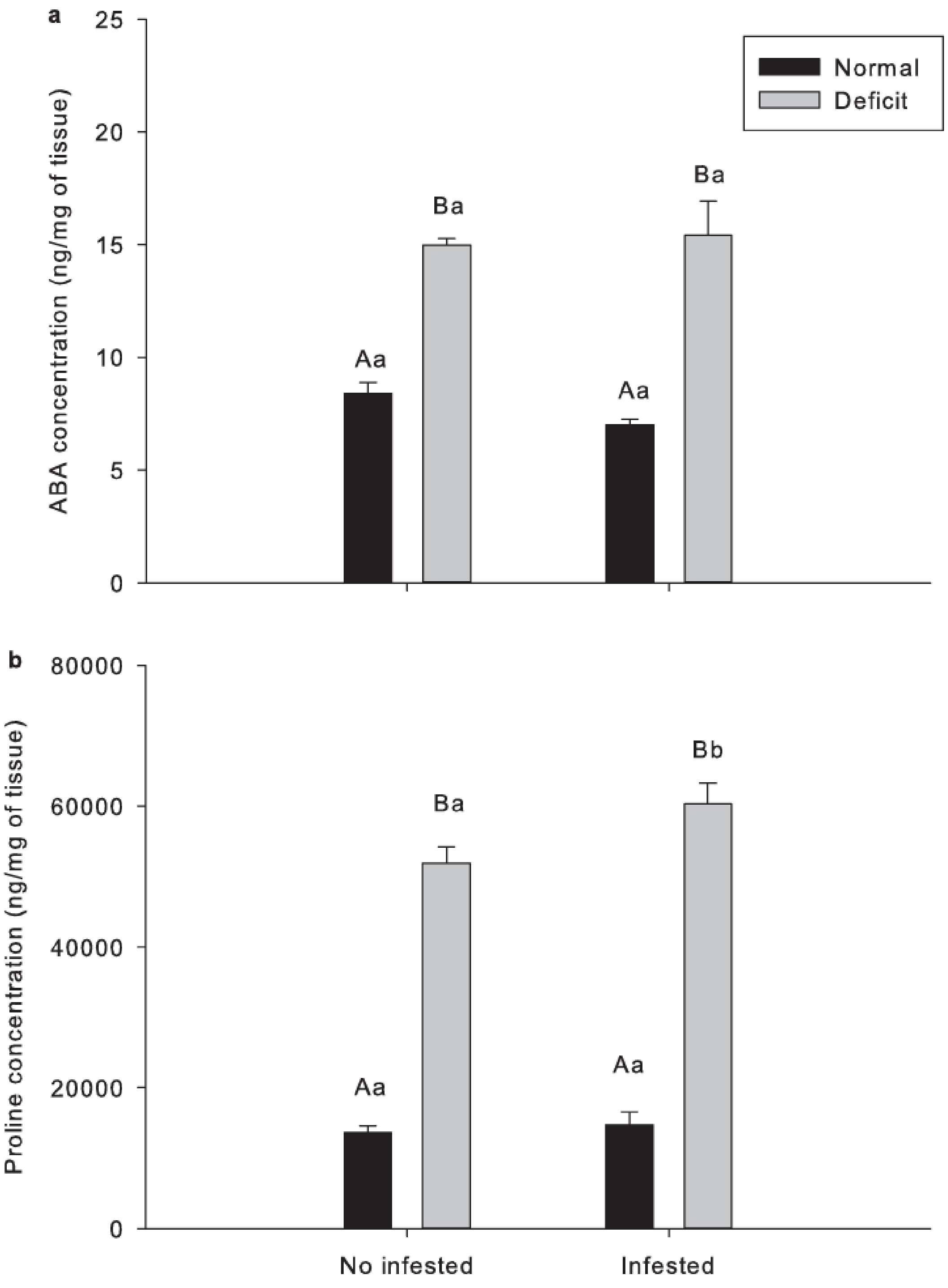


Figure 2.3. Physiological phytorhomones ABA (a) and Proline (b) of tomato plants under deficit and normal irrigation environments and infested with *Tuta absoluta* (Meyrick, 1917) (Gelechiidae: Lepidoptera), indicating response to hydric stress. Bars (Mean ± SEM; n = 6) with same capital letter indicate no significant difference between irrigation environments and those followed by same lowercase letter no significant difference between treatments by Tukey test at 95% of confidence level.

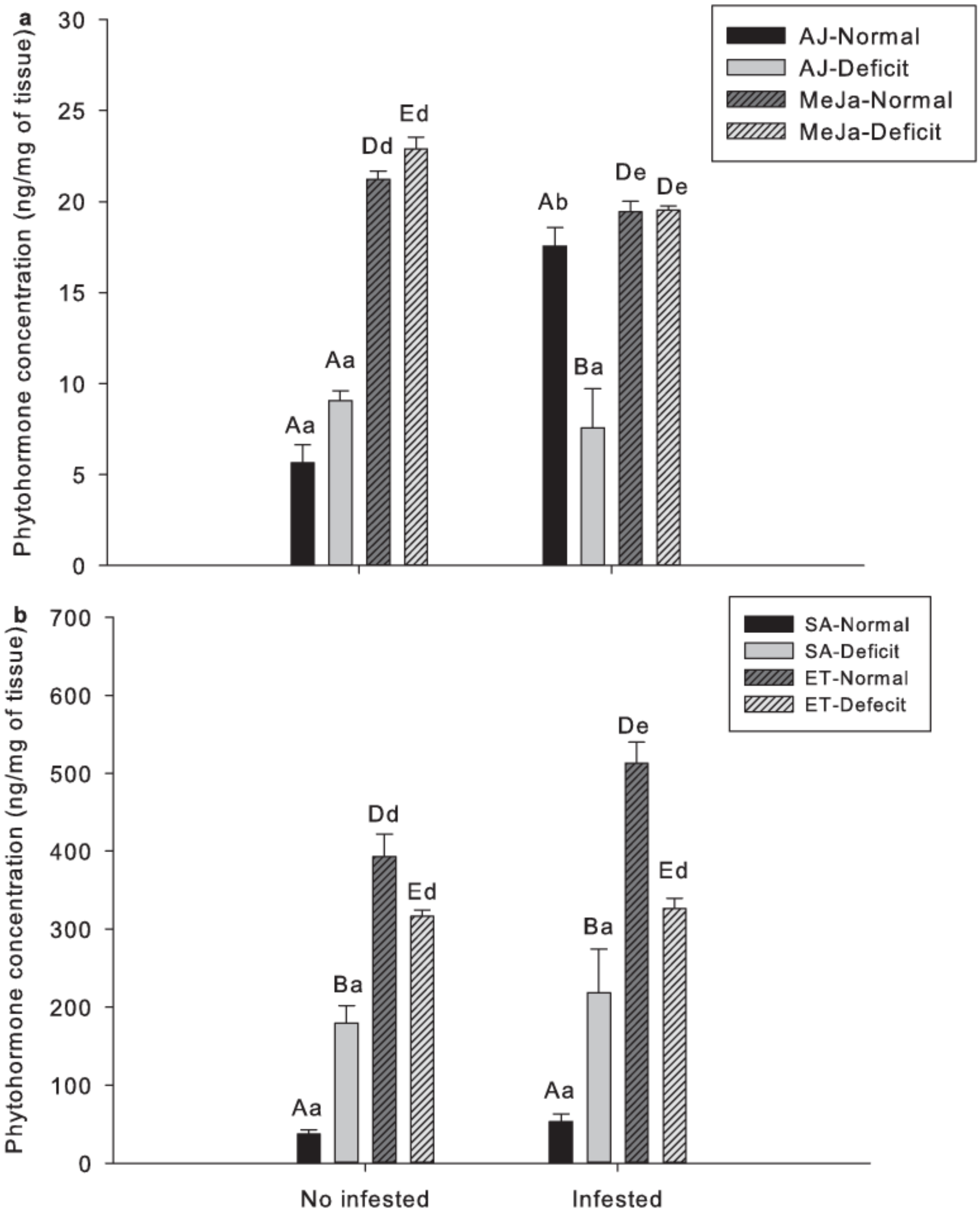


Figure 2.4. Physiological phytorhomones AJ and MeJa (a); SA and ET (b); of tomato plants under deficit and normal irrigation environments and infested with *Tuta absoluta* (Meyrick, 1917) (Gelechiidae: Lepidoptera), indicating defensive response. Phytohormone bars (Mean ± SEM; n = 6) with same capital letter indicate no significant difference between irrigation environments and those followed by same lowercase letter no significant difference between treatments by Tukey test at 95% of confidence level.

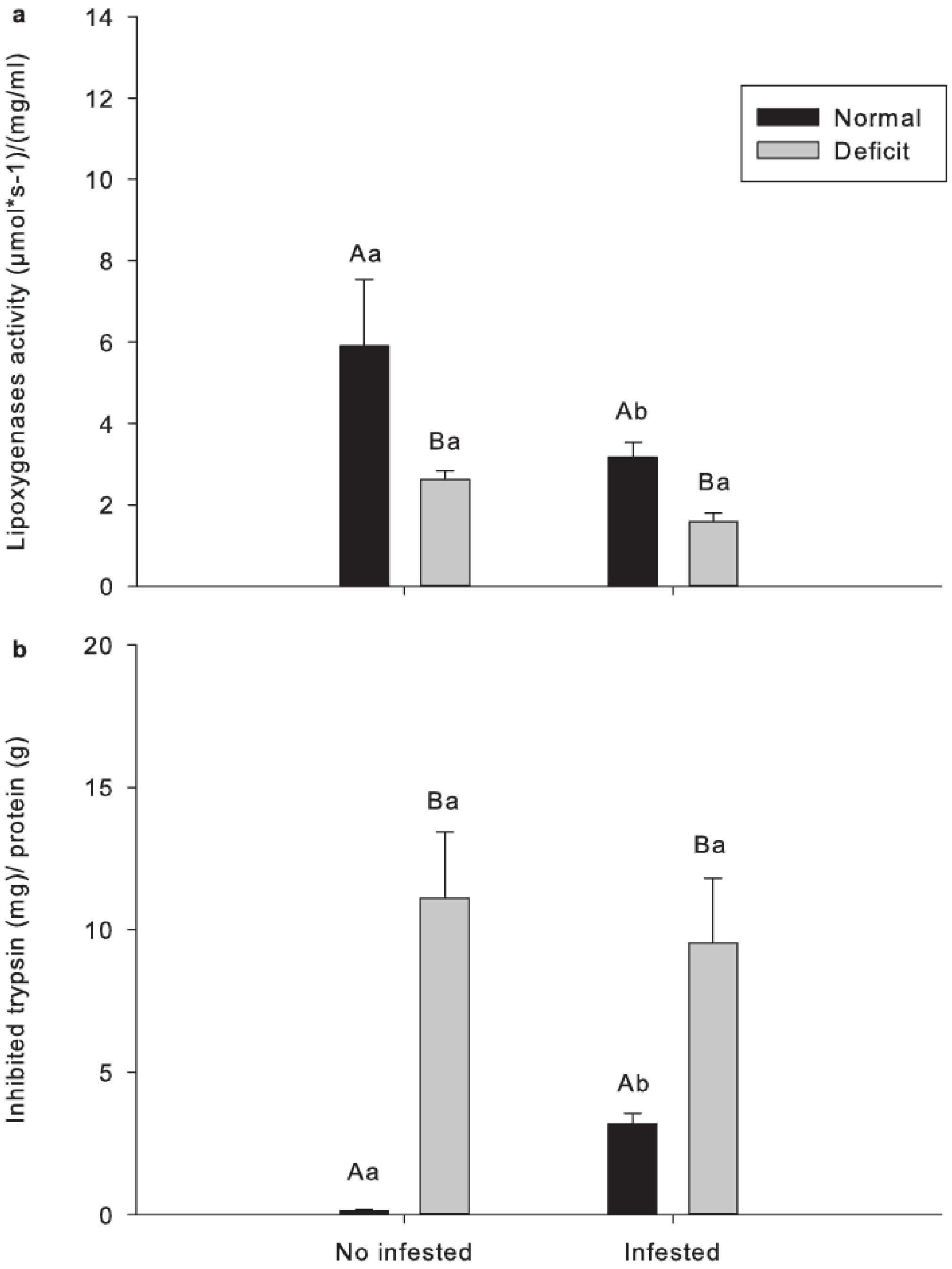


Figure 2.5. Defense response of tomato enzymes (a) Lipoxygenases and (b) Proteinase Inhibitors under deficit and normal irrigation environments and infested with *Tuta absoluta* (Meyrick, 1917) (Gelechiidae: Lepidoptera). Bars (Mean ± SEM; n = 6) with same capital letter indicate no significant difference between irrigation environments and those followed by same lowercase letter no significant difference between treatments by Tukey test at 95% of confidence level.

CONCLUSÕES GERAIS

Este trabalho destaca os efeitos do estresse hídrico em interações multi herbívoro-tomate. O deficit hídrico e a herbivoria, mediam as interações das cascatas hormonais, resultando em diferentes expressões de compostos defensivos. O ácaro generalista *T. urticae* teve uma influência importante na indução de compostos bioquímicos no tomateiro tanto negativa (Prolina, ACC, LOX, PPO), como positiva (AIA, Zeatina, AJ, AS). Os herbívoros especialistas *T. evansi* e *T. absoluta* mediam a interação das respostas de defesa, principalmente quando compartilham plantas hospedeiras com outros herbívoros do que isoladamente. O deficit hídrico afetou a produção do tomateiro, sem influência da herbivoria, e poderia tornar as lavouras mais suscetíveis para às lagartas que perfuram os frutos.

Tomate sob deficit hídrico foi menos adequado para o desempenho de *T. absoluta* devido a que as injúrias geradas pelas lagartas induziram uma resposta defensiva mediada pelo JA com alta produção de PI, contraria a "hipótese da planta estressada" e mais acordo com a "hipótese da planta vigorosa". A sinalização e interações entre hormônios vegetais, mostra diferenças importantes entre as condições de irrigação e a herbivoria por *T. absoluta*. As respostas de defensa, mediadas por JA / ET foram inibidas pelo antagonismo ABA / AS, sob deficit hídrico.

CONSIDERAÇÕES FINAIS

- Poucas pesquisas têm mostrado efeitos do deficit hídrico em interações multi herbívoro-tomate em experimentos de campo aberto, o que dá relevância a este trabalho

- A sinalização e interações entre fito-hormônios vegetais mostra diferenças notáveis de acordo com as diferentes condições de irrigação e herbivoria. O deficit hídrico e herbivoria media interações hormonais como AJ/ACC-Etileno e ABA/AS em comparação com plantas bem irrigadas, resultando em diferentes expressões de compostos enzimáticos defensivos, o que prejudica o desenvolvimento de herbívoros como *T. absoluta*.

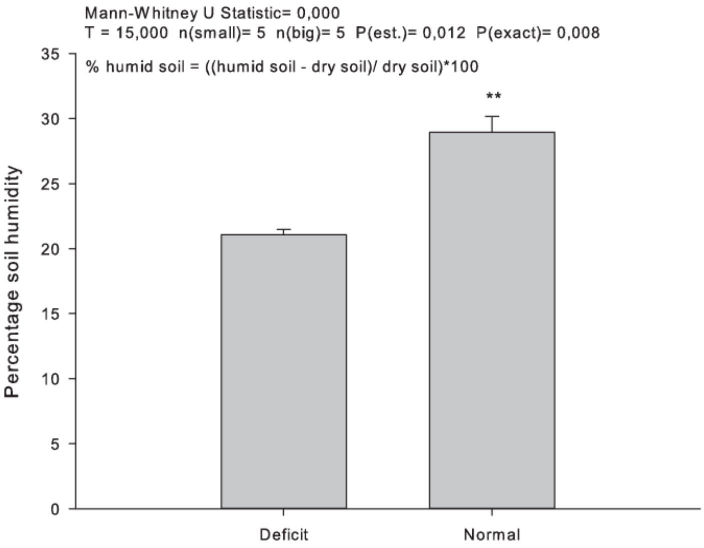
- O tipo de mecanismo de alimentação e especialização do herbívoro foi um fator importante na variação destas interações. Assim como a quantidade de espécies que podem-se alimentar numa planta simultaneamente.

- O deficit hídrico gera mudanças na produção do tomateiro em características físicas e quantidade de frutos, podendo levar as plantas a produzir maior número de frutos, mas de menor tamanho e peso e ao mesmo tempo poderia tornar as lavouras mais suscetíveis à pragas chave como a broca pequena do tomateiro.

SUPPLEMENTAL MATERIAL

SM1. Drip pipe and tutored system used in experimental plots

SM2. Comparison of soli humidity between deficit and normal irrigated plots



SM3. Equations

Crop Evapotranspiration (mm d^{-1}):

$$ETc = ETo \times Kc$$

$$Kc = (Kcb \times Ks) + Ke$$

Where: ETc = Crop evapotranspiration (mm d^{-1}); ETo = evapotranspiration reference (mm d^{-1}); Kc = crop coefficient; Kcb = basal culture coefficient; Ke = evaporation coefficient from the soil; Ks= stress coefficient.

Phytohormones concentration (ng of hormone / g of leaf tissue):

$$\frac{CPA \times EV}{STW}$$

Where: CPA = chromatograph peak area transformed by the calibrated linear regression; EV = extration solution volume; STW = sample tissue weight.

Lipoxygenases activity ((µmol.s^{-1})/(mg/ml)):

$$\frac{A234}{(\varepsilon \times l \times t)}\Big/P$$

Where: A234 = absorbance at 234 nm; ε = 25000 $M^{-1}.cm^{-1}$ (molar extinction coefficient of linoleic acid hydroperoxides at 234 nm); l = 1,0 cm (optical path) t= 150 seconds (reaction time); P = protein concentration (mg/ml).

Polyphenol oxidases activity ((µmol.s^{-1})/(mg/ml)):

$$\frac{A410}{(\varepsilon \times l \times t)}\Big/P$$

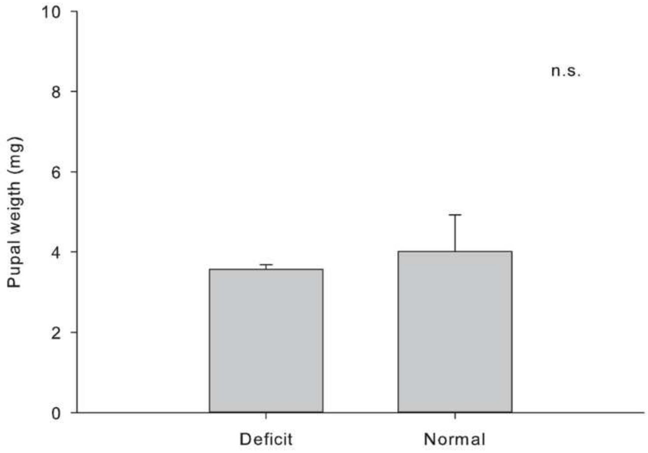
Where: A410 = absorbance at 410 nm; ε = 1470 $M^{-1}.cm^{-1}$ (molar extinction coefficient); l = 1,0 cm (optical path) t= 150 seconds (reaction time); P = protein concentration (mg/ml).

Proteinase inhibitors concentration (mg of inhibited trypsin / g of protein)

$$\frac{(A \times B)}{(C \times 1000 \times P)}$$

Where: A = control final absorbance less the sample final absorbance at 410 nm; B = sample dilution; C= trypsin factor 0,019 resulted from 1µL of active trypsin on substrate L-BApNA at 410 nm; P = protein concentration (g/mL).

SM4. Comparison pupal weight between deficit and normal irrigated plots



SM5. Insecticides and fungicides applied for preventive spraying before experiments:

Insecticide	Fungicide
Actara 250WG *Bemisia tabaci* type B, 20 g/100 L	Frowncide 500 SC *Phytophtora infestans*, 100 mL/100L
Cartap BR 500 *Neoleucinodes elegantalis*, 250 g/100 L *Tuta absoluta,* 250 g/100 L *Lyriomyza huidobrensis,* 250 g/100 L	Revus *Phytophtora infestans*, 60 mL/100L
Premio *Neoleucinodes elegantalis,* 20 mL/100L *Tuta absoluta,* 15 mL/100L *Lyriomyza huidobrensis,* 15 mL/100L	Daconil *Phytophtora infestans*, 200 g/100L *Septoria lycopersici*, 200 g/100L
Tiger 100EC *Bemisia tabaci*, 100 ml/100 L *Bemisia tabaci* type B, 100 ml/100 L	Cuprogarb 500 *Phytophtora infestans*, 300 g/100L *Alternaria solani,* 300 g/100L
Lannate *Neoleucinodes elegantalis,*100 mL/100 L	