

The Role of Methyl Jasmonate During the Biotic Stress in *Matricaria Chamomilla* L.

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Abstract

The objective of the present study was to evaluate the effect of methyl jasmonate (MeJA) in plant resistance to polyphagous thrips. Eight-weeks old diploid and tetraploid chamomile plants (*Matricaria chamomilla*) in stage of leaf-rosettes were pre-treated with 0.22 mM MeJA for 72 h and then co-cultivated with thrips for next 96 h. In both cultivars, there was an increase in the content of hydrogen peroxide and superoxide radical, with a maximum in plants after thrips infestation. The pre-treatment with MJ led to a reduction, but the measured values were still higher than those in the control plants. The accumulation of total phenols and flavonoids corresponded with the activity of phenylalanine ammonia-lyase (PAL). The highest PAL activity and the highest levels of total phenols were observed in plants infected with thrips. MeJA pre-treatment slightly decreased their values. Within the cultivars we noticed differences in the accumulation of monitored coumarins: (*Z*)- and (*E*)-GMCA, herniarin and umbelliferone. In diploids MeJA significantly ameliorated their accumulation caused by thrips. On the other hand, in tetraploids, changes were minimal. The amount of umbelliferone sharply increased in both cultivars which points to its role as a stress metabolite.

Highlights

- MeJA pre-treatment ameliorate negative effect of thrips
- thrips feeding enhanced PAL activity and coumarins content
- diploid cultivar reacted more significantly compared to the tetraploid one

INTRODUCTION

Various fungal, bacterial and parasitic diseases degrade the quality of important medical herbs and crops. During the long co-evolution plants have developed various defensive strategies. In addition to always expressed constitutive defence represented by physical (e.g. thorns, trichomes) or chemical (e.g. repellent or toxic chemicals) barriers, plants respond with a more specific induced defence. It is triggered specifically after an herbivore attack and thus allows plants to effectively manage their energy reserves via synthesis defence compounds only when needed. At the same time, volatile substances can act as attractants for herbivore predators (Mouden and Leiss 2021; War et al. 2012).

In general, feeding stimuli by chewing herbivores or certain phloem feeder are transduced via a signalling pathway mediated through the synthesis of jasmonate (JA) compounds (Lazebnik et al. 2014). Artificial manipulation of jasmonates induces the expression of JA-responsive genes, resulting in the formation of chemical and physical barriers in a pattern similar to herbivory. For example, triggering of synthesis of primary metabolites such as amino acids or sugars (Chen et al. 2020; Wei et al. 2021), secondary metabolites (Chen et al. 2020), enhancing antioxidant activity (Tianzi et al. 2018) or trichome density (Mouden et al. 2020). This state of preparedness for the attack of herbivores can subsequently manifest itself as a higher percentage of plant survival after the insect attack (Tierranegra-García et al. 2011), reducing oviposition or increasing larval and adult's mortality (Kawazu et al. 2013; Senthil-Nathan 2019).

In addition, some author suggests that application of volatile methyl jasmonate (MeJA) in inducing jasmonate response could be more effective than that of JA itself due to its high volatility and easy entry through stomata (Jang et al. 2014; Jiang and Yan 2018).

Echinothrips americanus is a polyphagous thrip species that in a very short time occupy large areas of plants. Their original territory was east part of North America and their first European catch was recorded in 1989. Since then, this species infested rapidly at least in 19 European countries. It can be easily recognized due to the white stripes pass across the chest and at the base of the wings. It has piercing-sucking mouthparts and feed on plant juices sucking from a wide range of host plants (Varga et al. 2010).

German chamomile (*Matricaria chamomilla* L.) belongs to a major group of cultivated medicinal plants. It owes its therapeutic activity to different groups of effective substances; such as sesquiterpenes, flavonoids, coumarins (herniarin and its glucosidic precursors (*Z*)- and (*E*)-2- β -d-glucopyranosyloxy-4-methoxycinnamic acid (GMCA); umbelliferone) and polyacetylenes (Franke and Schilcher 2012). Several papers, dealing with the changes of mentioned metabolites as a result of plant reaction to biotic stimuli, approved their involvement in plant defence processes (Repcak and Suvak 2013; Repcak et al. 2001). The results of previously published works (Repcak et Suvak 2013; Ducaiova et al. 2016) pointed out that the impact of phytophagous insects or/and at the same time MeJA increased the level of chamomile secondary metabolites. Since the used MeJA concentration (0.4 mM) significantly affected the accumulation of the total phenols and coumarins (Ducaiova et al. 2016), here we selected lower concentration, only 0.2 mM, for stimulation the defence processes. Based on these studies, we examined whether the exogenously added low concentration of MeJA before attack of *E. americanus* could ameliorate its negative impact. The induced stress intensity was evaluated at biochemical level by measuring of reactive oxygen species and total proteins content, as well as phenolic metabolites involved in defence responses, i.e. total phenolic and flavonoid content and coumarin content.

Polyploidisation, or genome duplication, is common in higher plants and is considered to be an adaptation mechanism which played significant role in plant speciation. Induces polyploidisation can influence overall plant physiological and biochemical processes and affect the biosynthetic pathways of primary and secondary metabolites (Madani et al. 2021). In several species the production of primary and secondary metabolites was higher in polyploids than in their diploid counterparts, even qualitative differences were displayed (Moetamedipoor et al. 2022; Park et al. 2021). Although overall enlargement of the plant organs was observed in chamomile in tetraploid cultivars, a clear correlation between the increase in the level of active compounds was not recorded (Repčák and Krausová 2009). However, under the impact of environmental stimuli or hormone treatment, the responses of diploid plants were more stimulated (Ducaiova et al. 2020; Petruľová et al. 2014). Therefore, the second aim of the present study was to evaluate defence responses of two chamomile cultivars differing in ploidy level (diploid vs. tetraploid).

MATERIAL AND METHODS

Plant cultivation and experimental design

Seeds of chamomile (*Matricaria chamomilla* L.), diploid cv. 'Novbona' and tetraploid cv. 'Lutea', were germinated in sterile sands. Three-week-old plants were transferred into plastic pots filled with garden substrate. Eight-week-old plant in stage of leaf rosettes were used for the experiment. The cultivation was held in a growing chamber (Fitotron®, Weiss Technik) under controlled 12-h photoperiod lighting conditions and temperature 25/20°C (day/night). The soil water-holding capacity was maintained to 60% with daily moistening. Thrips (*Echinothrips americanus* Morgan) were collected on the leaves of the *Alocasia macrorrhiza* in the greenhouse of the Botanical Garden using the exhaustor. Overall, four different variants were tested:

C – control plants sprayed with distil water

MeJA – plant treated with 0.22 mM aq. solution of MeJA

T – plant treated with 100 pieces of thrips for 96 hours

MJT – plant pre-treated with MeJA for 72 hours and subsequently treated with 100 pieces of thrips for the next 96 hours.

All plants were placed under the glass bells to prevent the spread of volatile MeJA and insect. A total of three glass bells with 8 plants were used for each variant. Whole leaf rosettes were used for all analyses. Damage caused by thrips feeding (hereinafter referred to as “silver damage”) was evaluated visually and expressed as total damaged leaf area in mm².

Quantification of stress-related parameters

Potassium phosphate buffer (50 mM; pH 7.0) homogenates were used for hydrogen peroxide and superoxide radical determination. The accumulation of hydrogen peroxide was estimated according to titanium chloride method (410 nm) and superoxide radical was estimated using hydroxylamine for the nitrite formation (530 nm). The amount of malondialdehyde (MDA) monitored by thiobarbituric acid reactive substance assay (TBARS) was used as an expression of the level of membrane lipid peroxidation (Dučaiová et al. 2016).

Quantification of total phenols, flavonoids and PAL activity

Total soluble phenols (TP) and flavonoids (TF) were extracted from fresh leaf rosettes with 80% methanol. Their content was determined spectrophotometrically at 750 nm using the Folin–Ciocalteu method (Ducaiova et al. 2016) with gallic acid as standard (Sigma-Aldrich) for TP, and at 420 nm using aluminium chloride method (Ordoñez et al. 2006) with quercetin (Sigma-Aldrich) as standard for TF.

The activity of phenylalanine ammonia-lyase (PAL) was measured as a production of *t*-cinnamic acid (*t*-CA) from phenylalanine in sodium borate buffer (Ducaiova et al. 2016). The HPLC conditions: column Kromasil SGX C18 7 µm (150 × 4.6 mm); flow rate 0.8 ml min⁻¹; mobile phase: 55% acetonitrile. Detection

was performed at 275 nm using *t*-CA standard compound (Sigma-Aldrich). The activity of PAL was expressed as nmol *t*-CA min⁻¹ mg⁻¹ proteins. Total soluble proteins were quantified in phosphate buffer extracts according to Bradford (1976) with bovine serum albumin (Bio Rad) as standard.

Quantification of secondary metabolites

The 80% methanol was used for extraction of secondary metabolites. The separation of coumarin-related compounds was performed using HPLC-DAD (Agilent Technologies 1260 Infinity device) on a reversed-phase column LiChroCart 4–4, C-18 (5 µm). The mixture of mobile phases A (water with 1% trifluoroacetic acid) and B (55% acetonitrile) was used in gradient programme with flow rate of 0.7 ml min⁻¹: 0–15 min 40% B; 15–25 min 100% B; 25–35 min 100% B; 35–40 min 0% B. A wavelength of 320 nm was used for quantitative analysis. For the separation of dicycloethers the mobile phase was changed to the mixture of A (acetonitrile:water:trifluoroacetic acid; 19:80:1) and B (90% acetonitrile) in gradient programme: 0–23 min 70% B; 23–25 min 0% B (Ducaiova et al. 2020).

Commercially available standards were used to construct calibration curves: herniarin (≥ 98%; Extrasynthese), umbelliferone (≥ 99%; Fluka), skimmin (Apin Chemicals), daphnin (≥ 95%; Extrasynthese), chlorogenic acid (≥ 98%; Fluka) and 1,5-dicaffeoylquinic acid (≥ 95%; Fluka). For commercially unavailable standards (*Z*) - and (*E*) -GMCA, 5-feruloylquinnic acid and dicycloethers isolated and purified substances with confirmed identity based on NMR analyses were used.

Statistical analysis

The comparison of differences in experiment was based on one-way analysis of variance (ANOVA) and Tukey's test at the significance level $p < 0.05$ (GraphPad Prism 9 Software, LLC, San Diego, CA, USA). Six individual plants mixed from three glass bells were used for analyses of each parameter. The whole experiment was repeated twice.

RESULTS

Effect of thrips on plant oxidative status

During the experiment, any significant increment or drop of biomass compared to the control plants was record ($0.11 \text{ g} \pm 0.003$ for both cultivars). MeJA treated plants exhibited mild leaves curling with prolonged isolation inside a glass bell. Exposure to thrips led to the visible changes in plants. On a closer study, numerous silver or brownish diffuse spots were observed, which in older leaves changed into the small necrotic lesions. This negative effect was in MJ pre-treated plants significantly reduced (Fig. 1).

In both cultivars, there was an increase in the content of hydrogen peroxide, with a maximum in plants after thrips infestation, more than 2-fold for diploid and 1.5-fold for tetraploid plants (Fig. 2). The pre-treatment with MeJA led to a reduction, but the measured values were still higher than those in the control plants. In the case of the superoxide radical (Fig. 2), a similar accumulation trend was observed. The highest values were found in T variants, 2-fold for diploid and 1.2-fold for tetraploid plants. In the diploid

cultivar, the addition of MeJA significantly reduced the level of superoxide radical, while in tetraploid plants the reduction was negligible. Changes in reactive oxygen species (ROS) accumulation led to a membrane damage only in the plants affected by thrips, where an approximately 1.3-fold increase in MDA content in both cultivars was observed (Fig. 2). The addition of MeJA reduced the content of MDA, but significantly only in diploid cultivar. Overall, the MDA content in tetraploid plants was approximately twice as high as in diploid ones.

The maximum values of total soluble proteins were recorded in T variants and MeJA pre-treatment reduced their values, but significantly only in case of diploid plants. Overall diploid plants showed higher values and subsequent increment was much more pronounced here, almost 2-fold.

Effect of thrips on phenol metabolism

The content of TP (Fig. 3) sharply increased and the maximum values were recorded in T variants, 1.6-fold increase for diploid and 2-fold increase for tetraploid plants. A similar accumulation trend was observed for TF (Fig. 3). Overall, the addition of MeJA before the thrips ameliorate their negative impact only in case of TF, where there was a significant decrease, but the values were still higher compared to control values. The accumulation of TP and TF corresponded to the PAL enzyme activity (Fig. 3). The highest enzymatic activity was observed in plants infested with thrips, 1.8-fold for diploids and 1.3-fold for tetraploids. Pre-treatment with MeJA slightly decreased their values. Surprisingly, a significant drop in the activity after application of MeJA alone was monitored in both cultivars.

Overall, the values in tetraploid plants were lower compared to diploid ones. A more pronounced difference occurred in the case of flavonoids and PAL activity, where the values in MJT and T variants did not rise as rapidly as in the case of the diploid cultivar.

The effect of thrips and MeJA pre-treatment was variously reflected in the accumulation of coumarins and their glucosidic precursors (Fig. 4) in both cultivars. In diploid plants, thrips infestation resulted in a significant increment compared to control almost in all monitored coumarins, with exception for (Z)-GMCA where the increase was only modest but still significant. Pre-treatment with MeJA reduce elevated level, but significantly only in case of umbelliferone and its glucosidic precursor skimmin, 3.7-times and 3.2-times, respectively. MeJA alone increased the values of coumarins, but their levels still remained lower in comparison to MJT and T variants.

The accumulation trend in tetraploid plants was similar to diploids, with exception for skimmin and (Z)-GMCA. Here, a significant increment over control values was observed, but with moderate differences between tested variants. Like in diploids, sharp, almost 2-fold increment was in case of stress coumarin umbelliferone.

Changes of three simple phenylpropanoids, namely chlorogenic acid, 1,5-dicaffeoylquinic acid, and 5-feruloylquinic acid, we monitored. The thrips invasion significantly raised their levels (Fig. 5). Pre-

treatment with MeJA reduced their levels, more pronounced in diploid plants in the case of 1,5-dicaffeoylquinic acid and 5-feruloylquinic acid, more than 1.5-times.

Effect of thrips on dicycloethers

As shown in Fig. 6, more enhanced accumulation of both (*Z*)- and (*E*)-isoforms of dicycloethers after thrips infestation was in the case of diploid plants, 3.5-fold and 1.8-fold, respectively. The protective effect of MeJA showed a significant decrease only in the case of (*E*)-isoform. In tetraploid plants, the increment was moderate, around 1.4-times for both isoforms. Here, in (*Z*)-isoform, the MeJA pre-treatment reduced its level to the control values.

DISCUSSION

The ability of plants to recognize and adequately respond to herbivores attack is essential for their survival. Important hormones in such induced resistance are JA or salicylic acid, which trigger many defence mechanisms that reduce herbivore survival, reproductive capacity, or preference for a plant. It has been well documented that exogenous applications of JAs can protect plants against herbivores by inhibiting or disturbing their normal development and reproduction processes, e.g. reduction in egg numbers (Shahabinejad et al. 2014), larval and pupal average weight (Jiang and Yan 2018) or fecundity (Tianzi et al. 2018). Even with increasing MeJA concentration, the total weight of larvae and adults and their survival time decreased (Senthil-Nathan 2019). These results suggest that pre-treatment of plants with JAs could provide a greater opportunity for plant survival via the activation of JA-responsive genes (Selig et al. 2016).

E. americanus represent phloem feeding insect whose infestation of piercing-sucking mouthparts is manifest as silver dots, which later turn into necrotic lesions. In our work, numerous silver or brownish diffuse spots were observed which were slightly reduced in MeJA pre-treated plants. Similarly, a foliar application of JA caused a marked reduction in silver damage caused by western flower thrips which had dose-dependent trend (Mouden et al. 2020) or locally-dependent trend (Chen et al. 2020).

Insect feeding activity provokes a local wound, and consequently triggers various metabolic responses driven by ROS formation. Apoplastic ROS burst acts as a first barrier against the pathogens and herbivores attack. Their elevated levels are not only toxic to the invasive organisms, but also to the plants themselves. Oxidative damage to significant biomolecules can lead to programmed cell death, which is part of a hypersensitive reaction (War et al. 2012). Among ROS, highly stabile and freely diffusible hydrogen peroxide may also act as secondary messenger by triggering the expression of defence genes and synthesis of various secondary metabolites (Fürstenberg-Hägg et al. 2013). Moreover, hydrogen peroxide has been found to have direct toxicity against insects (Maffei et al. 2007). In our experiment, we noted significantly higher ROS level after thrips invasion, which was ameliorated via MeJA pre-treatment. Similar results were also recorded in white cabbage infected with larvae of *Pieris brassicae* or *Phyllotreta nemorum* (Kovalikova et al. 2019) or in sorghum plants attacked by the butterfly *Chlio partellus* (Hussain

et al. 2014). The study of Tianzi et al. (2018) pointed out that MeJa-treated leaves of *Populus x euramericana* led to increased ROS generation, which was subsequently reflected in enhanced synthesis of antioxidant enzymes in *Clostera anchoreta* larvae fed on these leaves.

Overall, the characteristics of ROS accumulation can vary depending on the plant type and the invading insect. For example, in *Arabidopsis*, accumulation maximum of hydrogen peroxide after the oviposition of *P. brassicae* was after 72 hours (Little et al. 2006), while the attack of the phloem feeding aphid *Brevicoryne brassicae* showed changes after only 48 hours (Kunierczyk et al. 2008). In the case of *Bemisia tabaci*, no oxidative burst occurred even after 21 days of feeding (Kempema et al. 2006). In the aforementioned works, in parallel with the increasing concentration of ROS, an increased activity of not only antioxidant enzymes, specifically superoxide dismutase, catalase, peroxidase or polyphenoloxidase, but also other effective antioxidants, such as ascorbate and glutathione, was recorded. At the same time a number of corresponding genes associated with oxidative stress were reported to be upregulated (Kempema et al. 2006; Kunierczyk et al. 2008).

Malondialdehyde (MDA) represents a decomposition product of peroxidation of polyunsaturated fatty acid, and its concentration is usually correlated with the degree of membrane lipid degradation. Moreover, it has been suggested that elevated level of MDA may assist in the synthesis of more complex defence compounds and activate antioxidative enzymes. In addition, the emissions of leaf volatiles partially involved in indirect plant defence, are induced by lipid peroxidation after herbivore damage. Our results are in line with earlier reports, where MDA level was induced in cucumber plants infested with *Bemisia tabaci* (Zhang et al. 2008) or in rice plants attacked by *Cnaphalocrocis medinalis* (Chen et al. 2022). The rate of MDA formation can also depend on the insect type, e.g. scraping-sucking feeding activity is more stressful due to its concentration on large surface of plant tissues than the phloem-sucking insects which affect only vascular bundles (Kuster et al. 2022).

In addition to secondary metabolites, which are the main components of chemical defence, proteins also play an important role. Proteins are the main nutrient for insect growth and changes in their content can affect the overall physiological state of herbivores by reducing their growth rate, adult size or survival rate. In this study, a sharp increase in protein content was noted. Hussain et al. (2014) reported similar results, where their content increased even more after the addition of JA. Increased levels of proteins may reflect the plants' current need for antioxidant enzymes or other protein-based defensive compounds. For example, MeJA spraying on *Larix olgensis* seedlings significantly increased the bio-activities of superoxide dismutase, polyphenol oxidase and two proteinase inhibitors, which resulted in strongly reduced larval/pupal weights and survivals, as well as the fecundity of *Lamantia dispar* (Jiang and Yan 2018).

Among the secondary metabolites, plant phenols constitute one of the most common and widespread group of defensive compounds, which play a major role in plant resistance against herbivores, including insects. In plants, polyphenols are synthesized through the phenylpropanoid pathway in which L-phenylalanine is converted to cinnamic acid via PAL enzyme. Further, different enzymes at different levels

modulate the structure of the compounds formed at different levels of the pathway (Singh et al. 2021). PAL together with polyphenol oxidase have been considered as key enzymes in phenol metabolism, and their activity has been correlated to plant protection against insect feeding. In our study, the accumulation of phenols and flavonoids corresponded with the enzymatic activity of PAL with the maximum in plants attacked by thrips. Pre-treatment with MeJA slightly reduced PAL activity together with phenol levels. Similar results were also found in sorghum plants (Hussain et al. 2014), but on the contrary, an increased level of phenols and flavonoids was recorded in lettuce under the influence of MeJA and pill bugs (Tierranegra-García et al. 2011). Moreover, the level of activity may also depend on the type of plant cultivar. Aphid induced stronger enzyme elevation during the early period after infestation in resistant cultivars of chrysanthemum than to susceptible (He et al. 2011). Changes were also noted at the level of gene expression. For example, in pepper plants, the relative expression of PAL gene increased with prolonged invasion of western flower thrips (Zheng et al. 2019). Surprisingly, Selig et al. (2016) pointed out that the expression level can also be influenced by the type of insect itself, when phloem-feeder thrips upregulated and cell-content feeder aphid downregulated soybean PAL2 expression.

Among the phenolic acids, chlorogenic acid is the most abundant among the caffeoylquinic acid isomers in nature. It is considered as a defence metabolite in plants that provides protection against different herbivores via its oxidation to chlorogenoquinone which binds to free amino acids and proteins and reduced their digestibility. Higher accumulation of chlorogenic acid has been described in several plant species regardless of the feeding method, i.e. stem boring (Lee et al. 2017), piercing–sucking (Leiss et al. 2009) and chewing (Kundu et al. 2018). In these studies, anti-herbivore effect of chlorogenic acid was also confirmed by dietary tests, when a growth retardation, survival rate or larval weight reduction was detected. In our work, we noted an increase not only in chlorogenic acid, but also in 1,5-dicaffeoylquinic acid and 5-ferruloylquinic acid, both due to MeJA and thrips. Overall, the levels were higher in the case of the diploid cultivar, which, according to the conclusions of the study by Leiss et al. (2009), indicated that cultivars with higher values of the mentioned metabolites showed higher resistance. In addition to chlorogenic acid, other phenolic acids can also play an important role in protection. For example, exogenous application of cinnamic and vanillic acid negatively affected the oviposition and feeding preference of the larvae of *Achaea janata* and *Spodoptera litura* on castor plants (Usha Rani and Pratyusha 2014).

Among other phenolic metabolites we focused on coumarins, which content was significantly influenced by thrips infestation. Almost in all cases, pre-treatment with MeJA reduced their levels. A significant elevation not only due to the effect of thrips, but also due to the effect of MeJA itself was monitored in the work Repčák and Suvák (2013), so we can conclude that concentration (0.4 mM) used here could already have a stressful rather than acclimatizing effect. Increased levels of coumarins in chamomile plants were also observed under the influence of various abiotic, e.g. heavy metals (Kováčik et al. 2012), nitrogen nutrition (Sajko et al. 2018) or UV radiation (Petrůvová et al. 2014), and biotic stimuli, e.g. *Erypside cichoracearum* infection (Repčák et al. 2001).

Many phenolic metabolites, including coumarins, are toxic not only to herbivores, but also to the plants themselves. This negative effect is reduced by their sequestration into an inactive form in vacuoles, mostly by conjugation with glucose. With an increased need for active aglycones, the bond is broken by a specific glucosyltransferase (Singh et al. 2021). Occurrence of cellular membrane disruption, monitored here as an elevated level of MDA, should lead to a decrease in glycosidic form, GMCA and skimmin, and an increase in aglycones, herniarin and umbelliferone. Surprisingly, continuous increment in all mentioned coumarins was monitored here. Similar results were found in chamomile plants treated with salicylic acid (Ducaiova et al. 2013) or MeJA (Ducaiova et al. 2016).

The involvement of coumarins in defence processes suggests both their elevated concentrations in stressful conditions and also their antioxidant potential linked with the presence of conjugated system, ester bonds and with the number of bound hydroxyl groups. According to Lin et al. (2008), coumarins with higher amount of hydroxyl groups express better scavenging properties than unhydroxylated coumarins. From hydroxylated coumarins, we observed strong increment in the content of umbelliferone (7-hydroxycoumarin), but surprisingly, daphnetin (7,8-dihydroxycoumarin), was found only in trace amounts, but its glucosidic precursor daphnin (daphnetin-7-*O*-glucoside) significantly rose in MJT and T variants. The work of Petruľová-Poracká et al. (2013), points out that there are significantly higher concentrations of daphnetin in the anthodia than in the leaves.

Umbelliferone plays an important role, both as a stress coumarin and as a precursor for the synthesis of other coumarins (herniarin, esculetin or skimmin) (Chu et al. 2017). In this way, low amounts of umbelliferone under normal physiological conditions and elevated levels under stressful conditions were expected. Both in our experiments and in abovementioned works, a significant increase in umbelliferone was noted.

Polyacetylenes of spiroketal enol ether type are known secondary metabolites synthesized by Asteraceae plants. Together with other substances such as monoterpenes or sesquiterpenes, they are part of the volatile substances that create the typical aroma. In addition, they play an important role in plant defence thanks to their toxic properties, such as growth and reproductive inhibitors, repellents, and oviposition-deterrent (Minto and Blacklock 2008). Several studies showed that essential oils or substances alone showed strong larvicidal potential against mosquito (Ali et al. 2018; Govindarajan and Benelli 2016). The literature data about the changes of dicycloethers in chamomile plants during the stress conditions are expressed in two way, as their sum or as free form, so it is difficult to compare to our results. Similar to our results, an increasing trend was observed after biotic stress (Repčák et al. 2001). However, MeJA application alone resulted in different accumulation trends, an increase in the (*Z*)- isoforms and a decrease in the (*E*)-isoforms (Dučaiová et al., 2016). In the case of other abiotic stressors, a predominantly declining trend was observed (Repčák et al. 2001, Sajko et al. 2018).

An increased ploidy level not only leads to a higher accumulation of pharmaceutically important metabolites, but can also help better adaptation to environmental stimuli. Yang et al 2021 showed that plants with higher ploidy level produced more phenolic metabolites with allelopathic effects, which

allowed seedlings to survive better and colonize new habitats faster. The effect of ploidy level on the rate of attack by herbivores is not yet completely clear. Authors report both a lower (Nuismer et Thompson 2001) and a higher attack rate (Arvanitisa et al. 2010) in polyploid plants. Furthermore, it is known that polyploidy can cause several changes in physiology, morphology, and gene expression that can impact resistance to herbivores. For example, in Brassicales, genome duplication led to an increased rate of glucosinolate synthesis, which play a key role in defence against invading butterflies (Edger et al. 2015). Qualitative changes of terpenes in *Solidago gigantea*, namely an increase in sesquiterpenes in tetraploids and, conversely, an increase in monoterpenes and diterpenes in hexaploids, led to changes in the survival and growth of invading herbivores (Hull-Sanders et al. 2009a, b).

Due to the fact that in the case of our experiment diploid and tetraploid plants were separated, it is not possible to clearly determine the preference of invading thrips. Macroscopically observable manifestations of feeding in the form of silver and brown spots did not show significant differences in number between cultivars. Activation of defence mechanisms was manifested by increased accumulation of total proteins (possibly increased synthesis of antioxidant enzymes) and increased accumulation of coumarins and phenolic acids with potential antioxidant properties (chlorogenic acid, 5-dicaffeoylquinic acid, and 5-feruloylquinic acid). A higher content of phenolic metabolites in diploid *Centaurea phrygia* plants compared to tetraploid plants was also noted by Münzbergová et al. (2015), which led to increased resistance to insects. However, the total content of hydrogen peroxide and the rate of its increase was significantly higher in the case of diploid plants, which indicates a higher level of stress. Overall, we can say that diploid plants responded to stress to a higher extent, but the use of MeJA as a protective agent alleviated the subsequent insect-induced stress more significantly than tetraploid plants.

Declarations

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We the authors declare that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

The authors have no competing interests to declare that are relevant to the content of this article.

AUTHOR CONTRIBUTION

Conceptualization: Zuzana Kovalikova, Veronika Petrulova; methodology: Zuzana Kovalikova, Veronika Petrulova; data curation: Zuzana Kovalikova; writing—original draft: Zuzana Kovalikova; writing—review &

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Figures

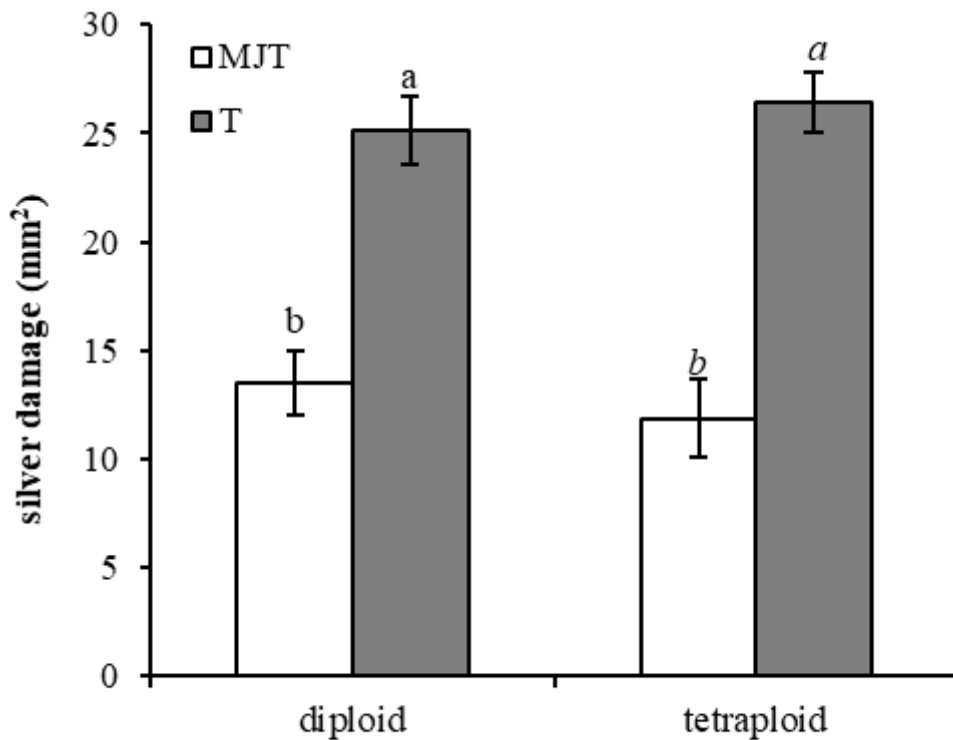


Figure 1

Feeding damage by thrips on chamomile leaf with or without MeJA pre-treatment. Data are means $\pm$ SDs ($n = 12$). Values within column, followed by the same regular or italic letter(s), are not significantly different according to Tukey's test ($p < 0.05$)

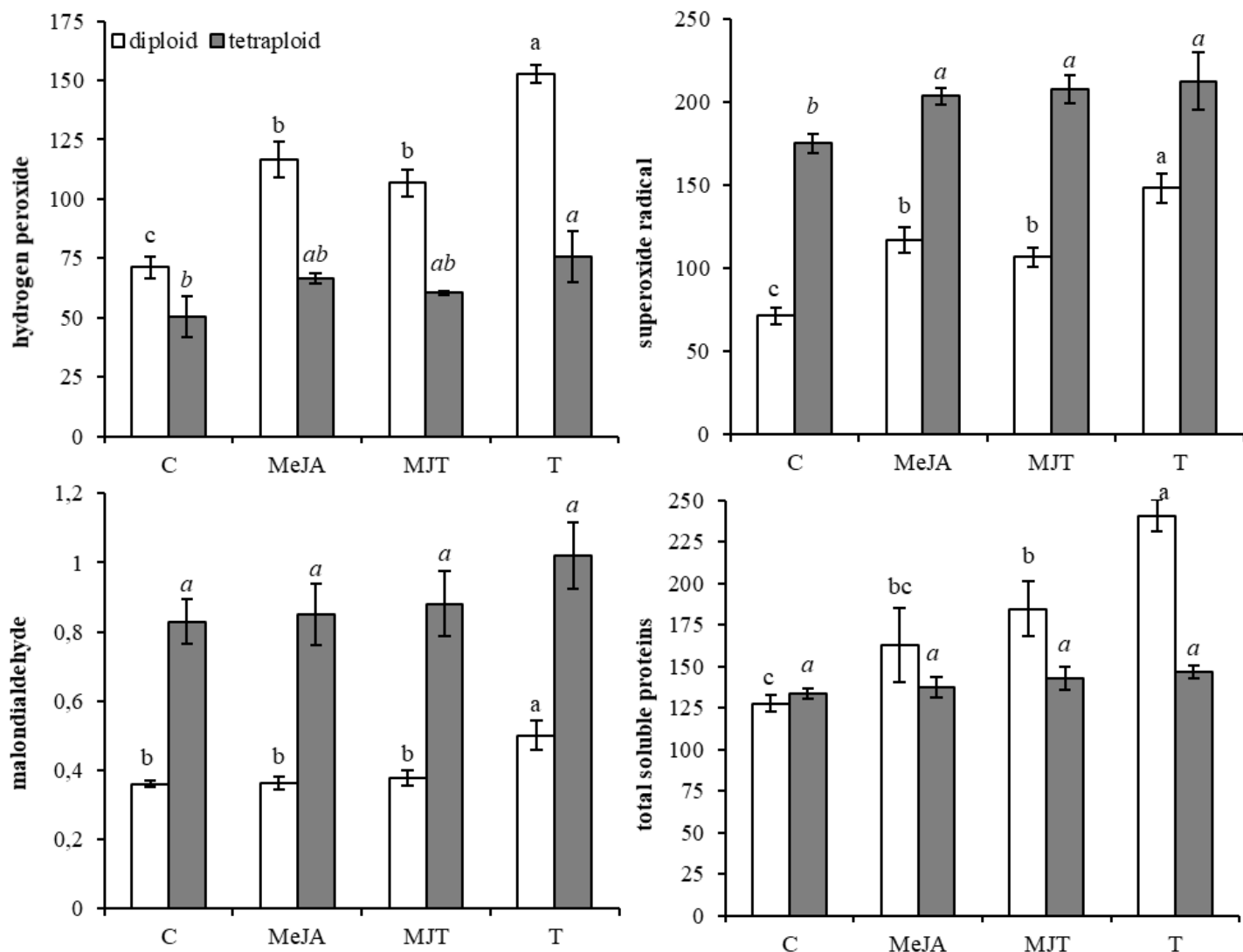


Figure 2

Effect of MeJA pre-treatment and thrips infestation on the content of hydrogen peroxide ($\mu\text{mol g}^{-1}$ DW), superoxide radical ($\mu\text{g g}^{-1}$ DW), malondialdehyde ($\mu\text{mol g}^{-1}$ DW), and total soluble proteins (mg g^{-1} DW) in chamomile leaf rosettes. Data are means $\pm$ SDs ($n = 12$). Values within column, followed by the same regular or italic letter(s), are not significantly different according to Tukey's test ($p < 0.05$)

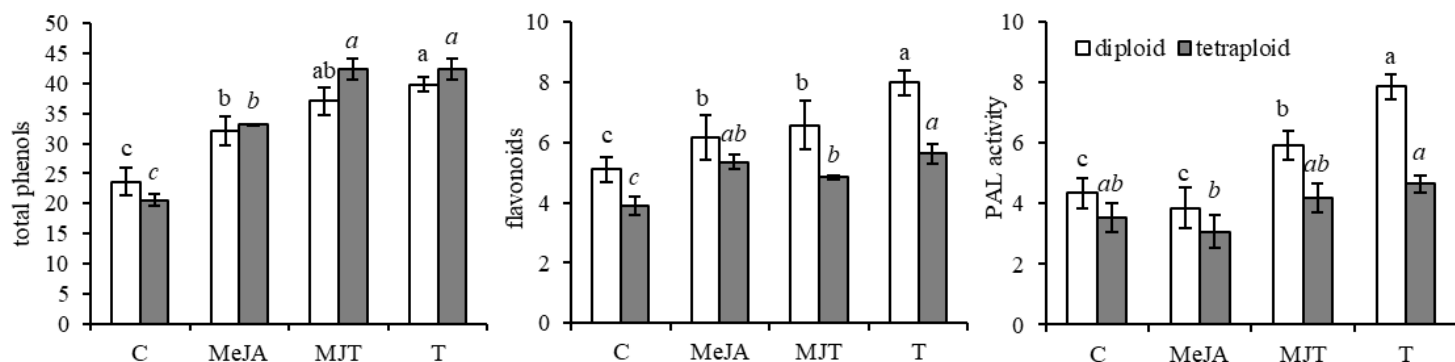


Figure 3

Effect of MeJA pre-treatment and thrips infestation on the content of total soluble phenols (mg g^{-1} DW), flavonoids (mg g^{-1} DW) and PAL activity ($\text{nmol } t\text{-CA min}^{-1} \text{ mg}^{-1} \text{ prot.}$) in chamomile leaf rosettes. Data are means $\pm$ SDs ($n = 12$). Values within column, followed by the same regular or italic letter(s), are not significantly different according to Tukey's test ($p < 0.05$)

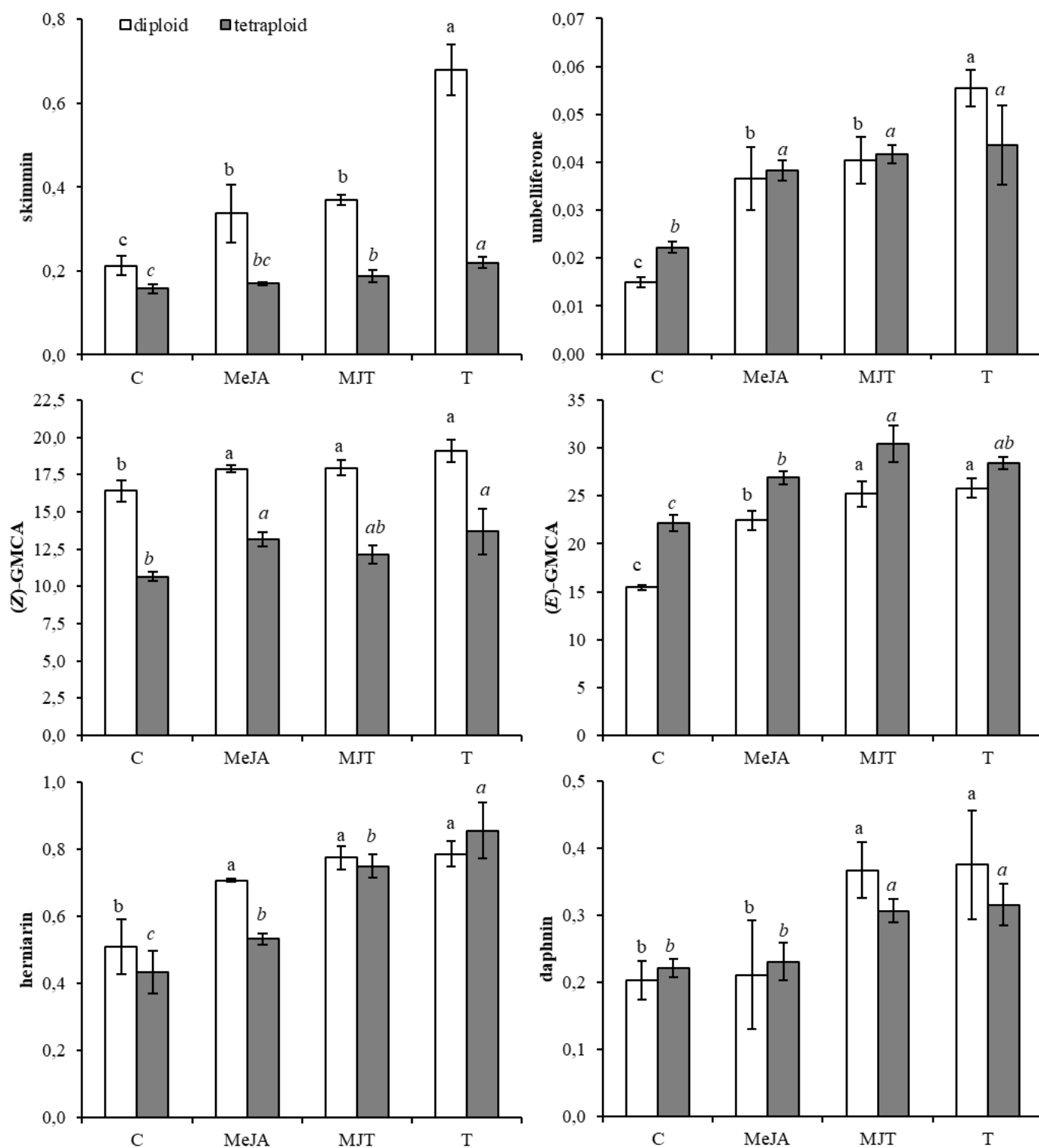


Figure 4

Effect of MeJA pre-treatment and thrips infestation on the content of coumarin-related compounds (mg g⁻¹ DW). Data are means $\pm$ SDs (n = 12). Values within column, followed by the same regular or italic letter(s), are not significantly different according to Tukey's test ($p < 0.05$)

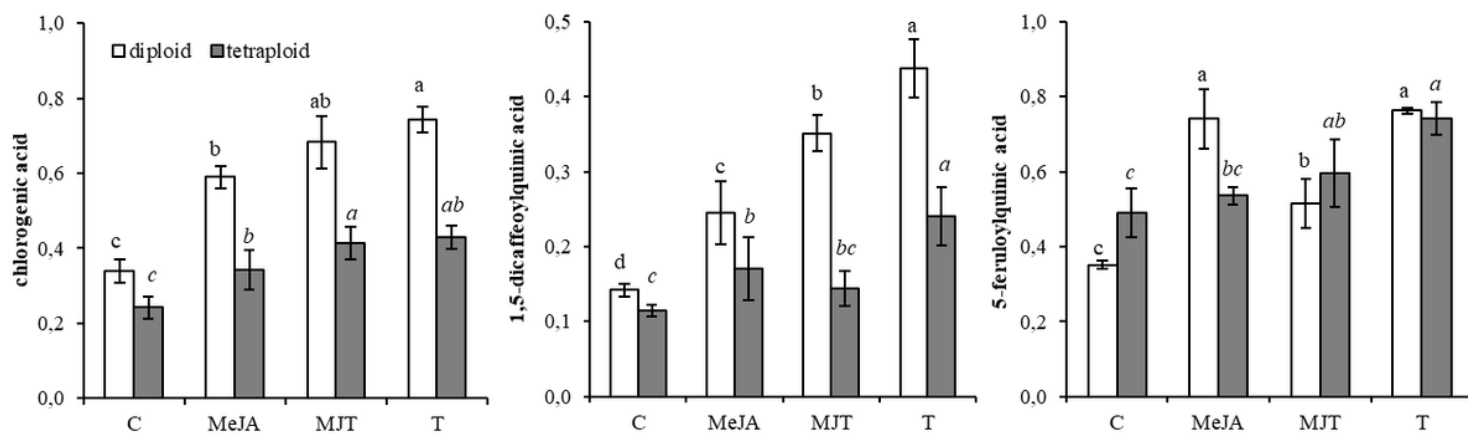


Figure 5

Effect of MeJA pre-treatment and thrips infestation on the content of simple phenylpropanoids (mg g⁻¹ DW). Data are means $\pm$ SDs ($n = 12$). Values within column, followed by the same regular or italic letter(s), are not significantly different according to Tukey's test ($p < 0.05$)

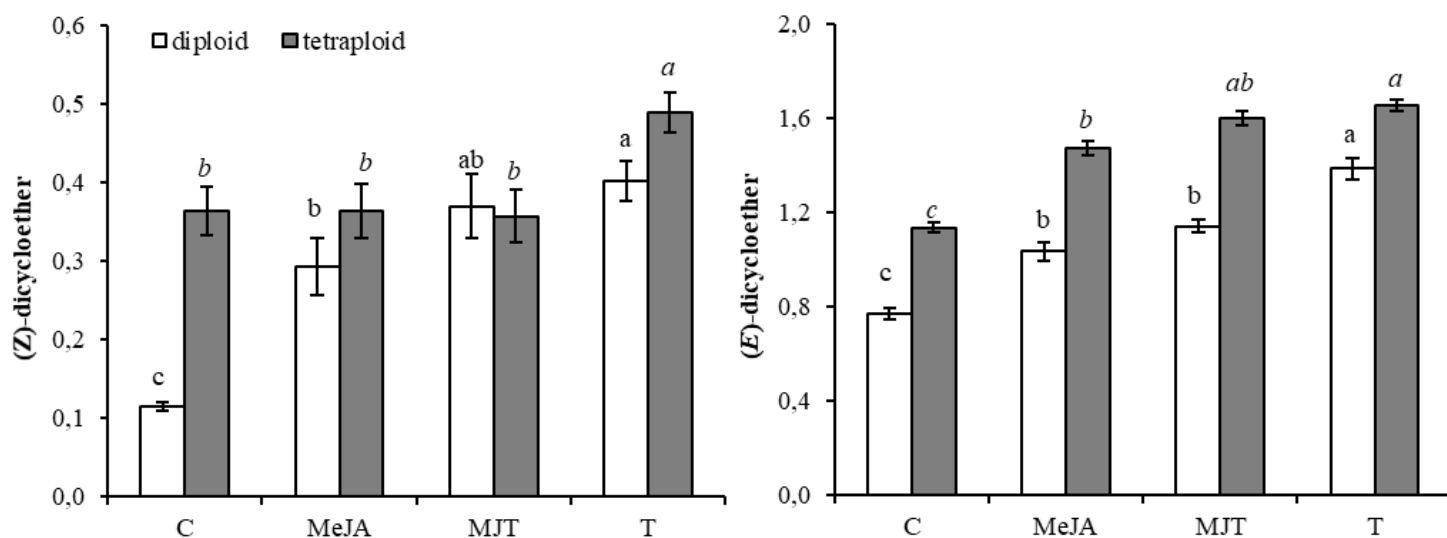


Figure 6

Effect of MeJA pre-treatment and thrips infestation on the content of dicycloethers (mg g⁻¹ DW). Data are means $\pm$ SDs ($n = 12$). Values within column, followed by the same regular or italic letter(s), are not significantly different according to Tukey's test ($p < 0.05$)