Defense of Host Plants against *Orgyia trigotephras* in Northeast of Tunisia

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ABSTRACT

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The egg-larval stage of *Orgyia trigotephras* were observed in shrubs maquis of Jebel Abderrahmane in north-east Tunisia, mainly on *Quercus coccifera* and *Pistacia lentiscus*, while only eggs were noticed on *Phillyrea media*. This kind of observation suggest us to study tree defense against *O. trigotephras* which will be explored by chemical analysis of *P. lentiscus*, *Q. coccifera* and *P. media*. Two types of analyses were the focus of this study to understand plant defense (i) primary metabolites and (ii) components of essential oils of these tested plants. Kjeldhal method was used for nitrogen and Mrssorr method for potassium, sodium and phosphorus extraction. Essential oils were extracted with the hexane solvent; chemical composition was determined using (GC/MS) methods. Oil compounds were identified by comparison to their retention time. Results of mineral extraction showed that percentage of potassium, sodium, phosphorus and nitrogen were more important in *P. lentiscus* and *P. media* than in *Q. coccifera*. Five major compounds were identified from essential oils of *Q. coccifera*, four from *P. media* and four from *P. lentiscus*. Nitrogen, which is a source of protein for insects, is produced in low concentrations in the foliage, decreasing nitrogen levels strategy for defending the plant against insect larvae. The absence of monoterpens in the foliage at *P. media* could explain the choice of larvae not to feed upon this host which probably confers resistance against this defoliator.

Keywords: Defense, essentials oil, minerals, *Orgyia trigotephras*, Tunisia

Plants, as a food source, have a considerable role in the dynamics of herbivorous insect populations due to their nutritive components (proteins, amino acids, carbohydrates, lipids, vitamins, minerals, water, etc.) and non-nutritional components (allelochemical compounds such as phenols, terpenes, glucosinolates, alkaloids, etc.) (Ohgushi 1992). Some chemicals substances released by infested plants are the result of mechanical disturbance of plant cells and therefore are not specific to the herbivore. However, other volatile products released as a result of damage

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are specific indicators for phyllophagous (Dicke et al. 1990).

The species *Orgyia trigotephras* (Lepidoptera, Erebidae) is distributed around the Mediterranean area, from Anatolia (Patočka and Turčáni 2008) to the Southwestern Europe, France (Bérard et al. 2010), Iberian Peninsula (Cifuentes 1997; Montoya and Masmano 1993) to the Northern Africa: Morocco (Villemant and Fraval 1993), Algeria (Rungs 1981) and Tunisia (Chénour 1955; Ezzine et al. 2010; Lord Rothschild et al. 1917).

Despite its polyphagia, *O. trigotephras* preferentially attacks oak species (Villemant and Fraval 1993). In Tunisia, larvae and egg batches of *O. trigotephras* were observed on *Pistacia lentiscus*, *Q. coccifera* (Ezzine et al. 2010), *Halimium halimifolium* (Ezzine et al. 2015), *Calicotome villosa*, *Erica multiflora* and *E. arborea* (Ezzine 2016). On *Phillyrea media*, only egg batches were observed (Ezzine 2016).

To highlight plant response to herbivore attack, we propose to study the defense mechanisms (direct and indirect) of *P. lentiscus*, *Q. coccifera* and *P. media* against *O. trigotephras*. In this work we aimed to (i) analyze secondary metabolites and (ii) estimate the importance of minerals of the tested plants.

MATERIALS AND METHODS

Study site.

This study was conducted using harvested biological material, from the outbreak site of *O. trigotephras*, in the northeastern of Tunisia (Jebel Abderrahmane, Cap Bon) in Delhiza (alt. 401 m, 36°51'N, 10°47'E) in 2009. The vegetation at the site is composed of Mediterranean maquis with 12 main plant species. Most abundant species were *Cistus crispus*, *C. villosus*, *Erica arborea*, *E. multiflora* and *Phillyrea media*. The two species *P. lentiscus* and *Q. coccifera* occurred at similar intermediate densities. Other plant species occurred only rarely (*Ampelodesmos mauritanicus* and *Chamaerops humilis*) or with low constancy (*Calicotome villosa*, *C. monspeliensis* and *Daphne gnidium*). Plant identification was carried out using plant guides (Schoenenberger et al. 1971; DGF 1995).

Collecting samples.

Leaves of *P. lentiscus*, *Q. coccifera* and *P. media* were collected in October 2009, dried in the shade and conserved for the chemical analysis: nitrogen (N), potassium (K), sodium (Na) and phosphorus (P) and the essential oil extraction.

Mineral extraction.

The total nitrogen was measured using the Kjeldhal method (Jackson 1958). This method required 3 steps: mineralization, distillation and titration. The percentage of nitrogen was obtained by the following equation:

$$N (\%) = 0.7 \times n/w \times 100,$$

with 1 ml of HCl N/20, n: quantity ml of HCl added to the sample and w: the weight of the vegetable powder (w = 100 mg). The assay was performed according to Mrssorr's method, ammonium phosphovanadomolybdate (Bray and Kurty 1945). The rate of each compound was calculated by the following equation:

$$\text{Compound rate } (\%) = (RS \times Cs / Rs \times V / Ws\ 1000) \times 100,$$

with RS: reading of the sample, Rs: reading of the standard, Cs: concentration of the standard, V: volume of the sample (50 ml) and Ws: weight of the sample (500 mg).

Essential oils extraction.

Dried and prepared leaves were crushed in a mortar containing liquid nitrogen until obtaining a powder. An amount of 1 g of powder was mixed with 10 ml of absolute methanol. Essential oils were extracted with the hexane solvent (Ressoug et al. 2005). The content of obtained essential oils (EOs) was dried over anhydrous sodium sulphate, and stored at -4°C until analysis. Assessment of the chemical composition of plant species EOs was carried out by gas chromatography/mass spectrometry (GC/MS) methods (Messaoud et al. 2005). The GC-MS unit consisted of a Perkin-Elmer Auto-system XL gas chromatograph, equipped with HP INNOWAX capillary column (Agilent 6280, 30 m × 0.25 mm, film thickness 0.25 µm) and interfaced with PerkinElmer Turbo mass spectrometer (Software version 4.1). The operating conditions were as follows: the injector temperature was 250°C; carrier gas was helium at 2 ml/min; a volume of 2 µl of each sample was injected in split mode; ion source temperature was 280°C. The temperature gradient started at 50°C, raised to 220°C (8°C/min), then to 220°C (10°C/min). Oil components were identified by comparison to their retention indices determined with reference to a homologous series of C_9-C_{24} of n-alkanes with those of authentic standards (Koroch et al. 2007). Identification was confirmed by comparison of their mass spectra with those recorded in the NIST08 and W8N08 libraries.

Data analysis.

The statistical analysis was performed using the SPSS-10.0 software package for Windows. Average of the different quantity of mineral composition were reported as mean percentage. Results were statistically evaluated by using analysis of variance (ANOVA) and complemented by multiple comparisons of means by the SNK test (Student-Newman-Keuls) at 95% confidence interval ($P < 0.05$). Results were expressed as mean ± standard error of mean (MSE).

RESULTS

Mineral analysis.

Results of mineral extraction was significant between the three plant species with ($F_{(2,6)} = 12.777$, $P = 0.007$) for phosphorus, ($F_{(2,6)} = 6.883$, $P = 0.028$) for potassium and ($F_{(2,6)} = 5.645$, $P = 0.042$) for sodium. The percentage of phosphorus (P), potassium (K) and sodium (Na) was more important in *P. lentiscus* (3.28 ± 0.187 10^{-5}%, 0.69 ± 0.012% and 0.72 ± 0.02%, respectively) and *P. media* (2.95 10^{-5}%, 0.63% and 0.53%, respectively) than in *Q. coccifera* (Fig. 1). Regarding the nitrogen concentration, there was no difference between the three plant species analyses ($F_{(2,6)} = 3.618$, $P = 0.093$). It was 2.13 ± 0.08% for *Q. coccifera* and 2.59 ± 0.181% for *P. media* (Fig.1).

Essential oil contents and composition.

The monoterpenes and sesquiterpenes were observed in *Q. coccifera*, *P. lentiscus* and ranged respectively from 14.19% and 95.64%. The diterpenes were observed in all species, with a low rate on *P. lentiscus* (2.03%). The triterpenes were observed in *Q. coccifera*, and *P. media* with respectively 50.81% and 63.4% (Table 1).

Five major compounds were identified from EOs of *Q. coccifera*, four from *P. media* and four from *P. lentiscus*. Betulinic acid, Betulin, β-Sitosterol and Sitost-4-en-3-one were in common between *Q. coccifera* and *P. media* (Table 1).

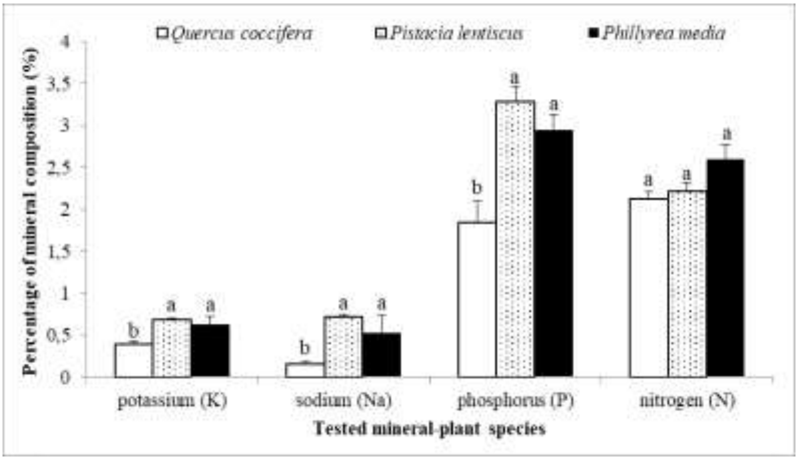

Fig. 1. Mineral compound (K, Na, P, N) rates of *Quercus coccifera*, *Phillyrea lentiscus*, *Pistacia media*. Each value represents the mean of three replicates ± SE. Values with different letters are significantly different at $P < 0.05$.

Table 1. Rate of the major essential oil compounds in the three tested species *Quercus coccifera*, *Phillyrea media* and *Pistacia lentiscus*

Compounds	RI	*Q. coccifera*	*P. media*	*P. lentiscus*
α-Pinene	938	tr	tr	4
α-Bisabolol	1581	7.69	tr	2.33
β-Caryophyllene	1366	tr	tr	12.85
Germacrene D	1418	tr	tr	11.67
Caryophyllene oxyde	1486	tr	tr	4.58
Betulinic acid	1674	4.22	9	tr
Betulin	1689	12.1	13	tr
β-Sitosterol	1718	5.1	6.8	tr
Sitost-4-en-3-one	1764	9.22	12	tr
Monoterpenes and Sesquiterpenes (%)		14.19	0	95.64
Diterpenes (%)		27.87	36.56	2.03
Triterpenes (%)		50.81	63.4	0
Total (%)		92.87	99.96	97.67

DISCUSSION

Plants and insects co-evoluate together, both have evolved strategies to avoid each other's defense systems (War et al. 2012). Direct defenses such as the production of toxic chemicals kill or retard the development of the herbivores (Hanley et al. 2007). In our work, we observed high rates of monoterpenes and sesquiterpenes in *P. lentiscus* that may have repellent effect on larvae of *O. trigotephras*. In fact, defensive components of the plant may affect the fitness and behavior of the herbivores (War et al. 2011). Studies conducted by Ezzine et al. (2010) on larvae of *O. trigotephras* reared on *Q. coccifera* and *P. lentiscus* showed that larvae have a relatively long development time and adults are relatively small when larvae feed on *P. lentiscus*. Furthermore, egg batches contain a high proportion of unfertilized eggs. Contrary to *Q. coccifera*, the lentisk is not favorable for the development of *O. trigotephras*.

Staudt et al. (2001) showed that attack of *Q. ilex* by larvae of the Erebidae, *Lymantria dispar* induce the emission of volatile organic compounds (VOCs). In fact, the stress induced by the emission of VOCs by attacked plants allows the release of reactive molecules (sesquiterpenes and monoterpenes) (Staudt and Lhoutellier 2007). The induced VOCs have a direct and protective role by dissuading defoliators and indirect, by attraction of their natural enemies (Kessler and Baldwin 2001; Thaler et al. 2001). The absence of monoterpenes in *P. media* can explain the no-choice of larvae of *O. trigotephras* to this species. Nitrogen reduction can be a defense strategy against herbivores (Feeny 1976). Nitrogen, as a source of protein for insects, is produced in low concentrations in the foliage of the host plant (Slansky and Feeny 1977). Casotti and Bradley (1991) showed that attacked foliage of *Eucalyptus* species may decrease its nitrogen concentration. Ezzine (2016) showed that larvae of the 1st, 2nd and 3rd instars of *O. trigotephras* imperatively feed on kermes oak; mature larvae feed on other shrub species mainly *P. lentiscus*. In years of high population density (2009 and 2014), most late-instar larvae of *O. trigotephras* moved to *P. lentiscus*, possibly due to the previous high exploitation of *Q. coccifera* by young larvae (Ezzine et al. 2015a) that may probably decrease the nitrogen concentration in the host plant. A rearing of larvae of *O. trigotephras* conducted by Ezzine (2016) on *Q. coccifera* and *P. lentiscus* showed that larvae pass through five larval stages. Contrariwise, on *P. media*, larvae pupate directly after the fourth instar, whereas this species contains a significant amount of nitrogen. Potassium and phosphorus are essentials for the physiology and the development of the insect (Dadd 1985; Mattson and Scriber 1978). Research conducted by Daryaei et al. (2008) on *L. dispar* showed that physico-chemical characteristics of clones of *Populus* × *Euramericana* have a considerable effect not only on the choice of the insect but also on nutritional indexes. Clones of *Populus* are chosen because of the important quantity of nutrients: 2.5% of nitrogen, 0.25 to 0.41% of phosphorous and 0.5 to 1.5% of potassium. The same thing was observed for *O. trigotephras*; the important quantity of these compounds on *Q. coccifera* and *P. lentiscus* allowed a good development of larvae. Ezzine et al. (2015a) showed that levels of defoliation of *Q. coccifera* by larvae of *O. trigotephras* reached almost 100% during the outbreak peak (2009) while they were always lower for *P. lentiscus*. Moreover, a test choice of larvae of *O. trigotephras* released in the lab on *Q. coccifera*, *Q.*

suber, *P. lentiscus*, *P. media*, *C. monoplienisis*, *E. multiflora* and *D. gnidium* showed that larvae choose the two oak species to feed (Ezzine 2016). Thus, kermes oak seems to be crucial for the development of larvae of *O. trigotephras*. Plants that do not allow a good development of caterpillars have a better resistance against defoliator, as observed for *P. media* which did not allow a good development of larvae (low nutritional indices) and caused a high level of larval mortality (Ezzine et al. 2014). In the field, no larvae were observed on *P. media*; it seems that the secondary metabolites (toxins) confer a resistance for the plant. The benefit of a secondary substance, in terms of increased adaptative fitness in the presence of phytophagous pests is related to the decreased fitness due to production, transport, storage and use of secondary metabolites (Feeny 1976).

The study of plant/insect interactions must integrate the 3rd trophic level (predators and parasitoids) as indirect defenses against insects (Price et al. 1980) mediated by the release of a blend of volatiles that specifically attract these natural enemies of the herbivores (Arimura et al. 2009). In fact, Harborne (1993) showed that each shrub species develops a chemical defense with which an entomofauna is associated and specialized.

RESUME

Ezzine O., Chograni H., Dhahri S. et Ben Jamâa M.L. 2020. Défense des plantes hôtes contre *Orgyia trigotephras* dans le nord-est de la Tunisie. Tunisian Journal of Plant Protection 15 (2): 81-89.

Les stades ovo-larvaires d'*Orgyia trigotephras* ont été observés dans le maquis de Jebel Abderrahmane dans le nord-est de la Tunisie, principalement sur *Quercus coccifera* et *Pistacia lentiscus*. Sur *Phillyrea media* seulement les pontes ont été notées. Ce type d'observation nous a incité à étudier la défense de la plante contre *O. trigotephras* qui sera examinée par l'analyse biochimique de *P. lentiscus*, *Q. coccifera* et *P. media*. Deux types d'analyses ont fait l'objet de ce travail pour comprendre la défense de la plante (i) les métabolites primaires et (ii) les composés des huiles essentielles de ces 3 espèces testées. La méthode Kjeldhal a été utilisée pour l'extraction de l'azote, celle de Mrssorr pour l'extraction du potassium, du sodium et du phosphore. Les huiles essentielles ont été extraites avec le solvant hexane; l'évaluation de la composition chimique a été réalisée par GC/MS. Les composés des huiles ont été identifiés par rapport à leur temps de rétention. Les résultats de l'extraction des minéraux ont montré que les pourcentages de potassium, de sodium, de phosphore et de l'azote étaient plus conséquents pour les espèces *P. lentiscus* et *P. media* que pour *Q. coccifera*. Cinq composés majoritaires ont été identifiés dans les huiles essentielles de *Q. coccifera*, quatre dans *P. media* et quatre dans *P. lentiscus*. L'azote, qui est une source de protéines pour les insectes, est produit en faibles concentrations dans le feuillage; la réduction de l'azote dans la plante hôte pourrait être une stratégie de défense développée par la plante contre les agressions des larves de cet insecte. L'absence des monoterpènes dans le feuillage de *P. media* explique le choix des larves de ne pas se nourrir sur cette espèce ce qui lui confère probablement une résistance contre ce défoliateur.

Mots clés: Défense, huiles essentielles, minéraux, *Orgyia trigotephras*, Tunisie

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ملخص

الزين، ألفة وهنية شقراني وسمير الظاهري ومحمد لحبيب بن جامع. 2020. دفاع النباتات العائلة ضد الحشرة *Orgyia trigotephras* في شمال شرق تونس.

Tunisian Journal of Plant Protection 15 (2): 81-89.

لوحظت يرقات وبيض *Orgyia trigotephras* علي الأحراش في جبل عبد الرحمان في شمال شرق تونس بشكل رئيسي على الكشريد (*Quercus coccifera*) والذرو (*Pistacia lentiscus*). على القتم (*Phillyrea media*)، وجد بيض فقط. دفعنا هذا النوع من الملاحظة إلى دراسة دفاع النبات ضد *O. trigotephras* التي سيتم فحصها من خلال التحليل الكيميائي الحيوي لنباتات *P. lentiscus* و *Q. coccifera* و *P. media*. نوعان من التحاليل كانا موضوع هذا العمل لفهم دفاع النبات هما (i) المستقلبات الأولية و (ii) مركبات الزيوت الأساسية لهذه الأنواع الثلاثة التي تم اختبارها. استخدمت طريقة Kjeldhal لاستخلاص النيتروجين وطريقة Mrssorr لاستخراج البوتاسيوم والصوديوم والفوسفور. تم استخلاص الزيوت العطرية بمذيب الهكسان. تم تقييم التركيب الكيميائي عن طريق تقنيات GC/MS. وتم تحديد مركبات الزيوت بالنسبة إلى توقيت الاحتفاظ لديها. أوضحت نتائج استخراج المعادن أن نسبة البوتاسيوم والصوديوم والفسفور والنيتروجين كانت أعلى في *P. lentiscus* و *P. media* مقارنة مع *Q. coccifera*. تم تحديد خمس مركبات رئيسية في *Q. coccifera* وأربعة في *P. media* وأربعة في *P. lentiscus*. يتم إنتاج النيتروجين، وهو مصدر بروتين للحشرات، بتركيزات منخفضة في أوراق الشجر، ربما كاستراتيجية دفاعية يطورها النبات العائل ضد يرقات هذه الحشرة. يمكن أن يفسر عدم وجود اليرقات بعدم وجود تربينات أحادية في أوراق *P. media* والتي من المحتمل أنها تمنح مقاومة ضد هذه الحشرة المزيلة للأوراق.

كلمات مفتاحية: تونس، دفاع، زيوت أساسية، معادن، *Orgyia trigotephras*

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