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Repellent activity of *Tanacetum parthenium* (L.) and *Tanacetum vulgare* (L.) essential oils against *Leptinotarsa decemlineata* (Say)

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Abstract

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is one of the most destructive pest species to have developed resistance to most chemical insecticides. We determined the composition and evaluated the potential of *Tanacetum parthenium* L. and *Tanacetum vulgare* L. (Asteraceae family) essential oil (EO) application as an alternative eco-friendly control strategy against *L. decemlineata*. We assessed the antifeedant activity for *L. decemlineata* larvae and adults by estimating the damage to potato leaves treated with three concentrations of EOs dissolved in ethanol (0.125, 0.25 and 0.5%). Results showed that *T. parthenium* EO was more effective against larvae, and *T. vulgare* was more effective against adults. In an olfactometer assay, the time required to choose an untreated leaf disc did not depend on the *Tanacetum* species, or life stage examined. However, the concentration of EO exhibited a significant effect on the behaviour of both developmental stages. At higher EO concentrations, both third instar larvae and adults require less time to choose an untreated leaf disc. Additionally, *T. parthenium* EO provoked more rapid movement away from the treated leaf disc than *T. vulgare*, especially at the highest concentration. Successful modification of *L. decemlineata* behaviour by the two *Tanacetum* oils suggests that they possess the potential for use in potato protection.

Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is an oligophagous insect that feeds on plants of the family Solanaceae, primarily on cultivated potato, *Solanum tuberosum* L. (Hare, 1990). Rapid food consumption, high fecundity and a remarkable adaptability to diverse environments make it one of the most destructive pests worldwide (Cingel *et al.*, 2016). The use of chemical insecticides is still the main strategy for *L. decemlineata* management. Populations of *L. decemlineata* have developed resistance to most pesticide chemistries (Alyokhin *et al.*, 2008; Huseth *et al.*, 2014; Brevik *et al.*, 2018). Due to concerns about the development of insecticide resistance and the human health consequences of insecticide exposure (Pandian and Ramesh, 2020; Upadhayay *et al.*, 2020), the search for alternative, eco-friendly strategies for managing *L. decemlineata* populations has intensified (Sablon *et al.*, 2012).

One strategy relies on the use of plant secondary metabolites that have coevolved as a defence mechanism against herbivorous insects (Isman and Miresmailli, 2011; Miresmailli and Isman, 2014). Secondary metabolites, including essential oils (EOs), can be extracted from aromatic plants belonging to Myrtaceae, Lauraceae, Lamiaceae and Asteraceae families, and represent complex mixtures of terpenes, terpenoids and aliphatic components (Isman and Miresmailli, 2011; Ntalli and Menkissoglu-Spiroudi, 2011; Regnault-Roger *et al.*, 2012; Pavela and Benelli, 2016; Chaubey, 2019). Many studies have reported repellent, antifeedant and insecticidal activities of EOs and other secondary metabolites, as well as their influence on various fitness and biochemical/physiological traits of insects (Szczepanik *et al.*, 2005; Isman, 2006; Pavela *et al.*, 2009; Rattan, 2010; Kim and Lan, 2011; Jumbo *et al.*, 2014; Kiran *et al.*, 2017; Tak and Isman, 2017). Insecticidal and antifeeding effects of plant extracts and EOs have been recorded in *L. decemlineata* (Kostić *et al.*, 2007; Gökçe *et al.*, 2012; Bekircan *et al.*, 2015). Compared to conventional insecticides, the use of EOs in pest management has several advantages. EOs contain many components with diverse mechanisms of action that slow down the evolution of physiological and/or behavioural adaptations in target organisms. A very small number of EOs and their dominant components are toxic to

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mammals (Isman, 2006). Due to the high volatility and low persistence and an outdoor half-life of less than 24 h, EOs are relatively safe for the environment (Regnault-Roger et al., 2012).

In the current study, we evaluated the use of EOs from *Tanacetum parthenium* and *Tanacetum vulgare* (Asteraceae family) in *L. decemlineata* control. These two perennial species are native to Eurasia and naturally occur in the flora of Serbia (Ranđelović et al., 2005). *T. parthenium* and *T. vulgare* EOs, as well as their dominant components, camphor and thujone, express strong biological activity against many harmful insects (Brewer and Ball, 1981; Hough-Goldstein and Hahn, 1992; Gabel and Thiery, 1994; Tsao et al., 1995; Larocque et al., 1999; Riddick et al., 2000; Pavlidou et al., 2004; Pavela et al., 2010; Szołyga et al., 2014; Czerniewicz et al., 2018; Devrnja et al., 2020). Treating potato leaves with extracts of *T. vulgare* decreased survival and retarded the growth of *L. decemlineata* larvae (Ploomi et al., 2006; Ertürk and Uslu, 2007).

In addition to the lethal effects of EOs, modification of behaviour at sublethal insecticide concentrations may also provide protection to crops (Hummelbrunner and Isman, 2001). For example, the push-pull control strategy uses repellent substances to move pests away from protected crops to traps and trap crops that can be treated with attractants (Cook et al., 2007). In a broad sense, repellency is related not only to movement away from an odour source, but also to the inability to find or recognize the host. According to this broad definition, repellent substances may provoke a behavioural response without direct contact or after direct contact with a pest (Deletre et al., 2016). Antifeedants that disrupt feeding behaviour pre- or post-ingestively, as well as compounds that inhibit host attraction, are considered to be repellents.

Investigations of the behavioural responses of *L. decemlineata* to *Tanacetum* oils are scarce. The study of Panasiuk (1984) pointed to avoidance behaviour of *L. decemlineata* adults in response to potato leaves treated with a single concentration of *T. vulgare* EO, while Kostich et al. (1993) confirmed an antifeedant effect of a single concentration of *T. parthenium* EO on second instar larvae. It has been previously demonstrated through olfactometer assays that starved female beetles will not feed on leaf discs treated with *T. vulgare* or *T. parthenium* EOs (Kostić et al., 2003).

In the current study we examined the repellent effects of three concentrations of *T. parthenium* and *T. vulgare* EOs in multiple different assays, including direct contact of third instar larvae and adults with *Tanacetum* oils. Our factorial experimental design (two *Tanacetum* EOs × two developmental stages × three EO concentrations) allowed for the exploration of the main and interaction effects of the three factors and addressed the following questions, as follows: (1) Are the repellent effects of *Tanacetum* EO species-, developmental-stage- and/or concentration-specific? (2) Does the sensitivity of *L. decemlineata* to increasing EO concentrations depend on the *Tanacetum* species and/or the developmental stage? (3) Do larvae and adults have different responses to *T. parthenium* and *T. vulgare* EOs?

Materials and methods

Collecting plant material and extraction of EOs

T. parthenium and *T. vulgare* plants in the full-blossom stage were collected from the location of Brezovica (42°13′19″N, 21°0′17″E; 400 km south of Belgrade, Serbia) and Aleksinac (43°33′0″N,

21°42′0″E; 206 km southeast of Belgrade, Serbia), respectively. *Tanacetum* species were identified according to Josifović et al. (1975). Above-ground (herbaceous) plant parts were air-dried at room temperature (22–25°C) for 7 days and used to obtain the EOs in a Clevenger-type apparatus (European Directorate for the Quality of Medicines, 2002). The extracted EOs were transferred into dark glass flasks, filled to the top and kept at 4°C until use. The extracts were dissolved in 96% ethanol to concentrations of 0.125, 0.25 and 0.5%.

Characterization of the EOs

Oil analysis was accomplished using gas chromatography (GC) and mass spectrometry (MS) analyses, as described by Block et al. (2006). GC analyses were performed using an HP-5890 Series II gas chromatograph with a split/splitless injector, fused silica capillary column (25 m × 0.32 mm), coated with non-polar stationary phase HP-1 (cross-linked methyl silicone, 0.5 µl film thickness) and a flame ionization detector. GC/MS analyses were performed on a Hewlett-Packard 5890 gas chromatograph directly coupled to a Hewlett-Packard HP 5971 A (70 eV) mass selective detector. Component identification in the tested samples was carried out with GCD ChemStation Software G1701BA version B.00.00 (Agilent Technologies, www.chem.agilent.com) using the probability merge search engine along with a Wiley 275 L mass spectrum database library (www.onlinelibrary.wiley.com), by comparing the MS of constituents with those from the Agilent MS library.

Collecting and rearing insects

L. decemlineata adults were collected at the location of Dobanovci (25 km west of the centre of Belgrade) in potato fields not treated with pesticides. In the laboratory, adults were placed in glass cylinders in which potato was grown under conditions optimal for the development of *L. decemlineata* ($T = 27 \pm 1$°C, RH = 60 ± 5%, neon diffuse light of intensity 30,159.29 cd under a 16:8 h light:dark regime). After eggs were laid on the potato leaf mass, the adult individuals were removed and egg hatching, larval moulting and eclosion of *L. decemlineata* adults were monitored. Identification of life stages was carried out according to Boiteau and Le Blanc (1992). After the moulting, larvae were transferred to new cylinders. Bioassays were performed on third instar larvae (L_3) 1 day after moulting and in adults 4 days after eclosion.

Antifeedant bioassay

Potato plants (cultivar Desiree) used in the bioassay were 6- to 7-week-old and 25–30 cm in height. The potato leaf mass was treated with ethanol solutions of EOs at three concentrations: 0.125, 0.25 and 0.5%; control potato plants were treated with 96% ethanol. Treatments were carried out by spraying with a TLC sprayer (Sigma-Aldrich). A total of 40 ml of solution per m^2 was used for the potato treatments. After a 15-min air-drying of plants at room temperature, six third instar larvae or six adults (three females and three males) that had been starved for 24 h were placed on leaves, and the plants with insects were covered with glass cylinders and transferred to a microclimate chamber (Danfoss EKH 20 operational system; $T = 27 \pm 1$°C, RH = 60 $\pm$ 5%, neon diffuse light of 30,159.29 cd intensity under a 16:8 h light:dark regime). After 48, 72 and 96 h of continuous exposure to treated leaves, leaf mass damage was estimated using a 0–10

scale (with $0 = 0\%$ and $10 = 100\%$ leaf mass damage). The bioassay was set up in five replicates per experimental group (one control and six groups treated with three concentrations of the two EOs). The antifeedant index (AFI) was calculated according to the formula:

$$AFI = (C - T)/C \times 100,$$

where C is the consumption of control leaves and T is the consumption of treated leaves (López-Olguín *et al.*, 1999).

Data were tested for normality of distribution by the Shapiro–Wilk test and for homogeneity of variances by Levene's test. Since leaf damage did not have a normal distribution, data comparisons between experimental groups were carried out by Kruskal–Wallis analysis of variance (ANOVA). To reveal whether the treatment groups differed significantly from the control group, the Kruskal–Wallis test was followed by the Bonferroni correction (McDonald, 2014). To estimate the main and interaction effects of the examined factors, non-parametric two- and three-way ANOVAs were performed according to Brunner and Puri (2001), followed by the Bonferroni test.

Avoidance of EO volatiles in non-contact and contact bioassays

A two-choice bioassay for evaluating the influence of EOs on host attraction was performed in an olfactometer. At the beginning of the bioassay *L. decemlineata* female adults were not in direct contact with treated leaf discs. The olfactometer was made of thick glass. The overall dimensions of the usable space in the olfactometer were $28 \times 15 \times 5$ cm (length × width × height). The air flowed from two entrances, through the expanded part of the olfactometer (15×7 cm) and three tunnels (16.5 cm long and 3 cm wide), to two exits on the opposite side. The expanded part of the olfactometer prevents air turbulence. The olfactometer uses an air pump and rubber-coated tubes (9 mm in diameter) for the inlet air to the manifold, the air flow regulator, the rotameter and the glass air hub with activated charcoal, which served to neutralize the odouriferous substances in the air. Leaf discs of potato were cut with a cork-borer (20 mm in diameter) and immersed for 3 s in ethanolic solutions of EOs at concentrations of 0.125, 0.25 and 0.5%. Control leaf discs were immersed in 96% ethanol. After air drying for 15 min, control and treated leaf discs were placed in the right and left tunnels, respectively. For the control group, leaf discs treated with ethanol were placed in both tunnels. Third instar larvae 1 day after moulting and 4-day-old females starved for 24 h were used in the experiment. In each trial, one individual was put in the olfactometer so that the distance between the potato leaf discs and female adults was 21 cm, while third instar larvae were 2 cm away from the disc. Choice time was recorded as the time needed to 'make the decision' and to move towards the control disc.

In the contact bioassay, the treated discs were placed in Petri dishes ($d = 9$ cm), and then one third instar larva that was starved for 24 h was placed on each leaf disc. The retention of larvae on the treated or control discs was monitored for 5 min and expressed as escape time, i.e. the time in s before a larva left the disc. None of the control larvae left the leaf disc within the observation period.

Both bioassays were set at 10 individuals per experimental group. Log-transformed values of adult choice times and larval escape times had a normal distribution (Shapiro–Wilk test) and homogeneous variances (Levene's test) and thereby satisfied the assumptions for parametric analyses. These data were analysed

by classic one- and two-way ANOVAs followed by a Bonferroni test. For comparison of the control with the treatment groups, the parametric Dunnett test was used.

Data on the larval choice time had nonhomogeneous variances and were analysed by Welch's ANOVA. Significant differences between the treatment groups and the control were revealed by the Games-Howell test followed by the Bonferroni correction (McDonald, 2014). Two-way and three-way non-parametric ANOVAs followed by the Bonferroni test (Brunner and Puri, 2001) were carried out to test the main and interaction effects of *Tanacetum* species, developmental stage and EO concentration.

Results

Composition of Tanacetum essential oils

The composition of the two *Tanacetum* oils is given in table 1. Thirteen compounds are present in both oils but in different concentrations. The major constituent of the *T. parthenium* oil was camphor while β-thujone was estimated at the highest concentration in *T. vulgare*. Comparison of constituents with concentrations greater than 1% in *T. parthenium* oil revealed that *T. parthenium* had a higher content of camphor, camphene, *trans*-chrysanthenyl acetate, bornyl acetate and α-pinene. The content of camphene, bornyl acetate and α-pinene in *T. vulgare* oil was below 0.5% but it contained β-thujone and 1,8-cineole that were not present in *T. parthenium*.

Antifeeding effects of Tanacetum EOs

Defoliation was significantly decreased in EO-treated groups when leaf tissue was exposed to third instar larvae, indicating a phagodeterrent effect of the applied *Tanacetum* oils (fig. 1). Significant differences among the experimental groups at the end of the observation period (96 h) were revealed ($H_{6,35} = 23.79$, $P = 0.0006$). After 96 h, both examined *Tanacetum* EOs provoked a significant reduction in leaf damage which was two-fold or fourfold lower in the treatment groups than in the control group (fig. 1). Leaf damage strongly depended on *Tanacetum* species and on the concentration of the EO (table 2). On average, higher EO efficacy was recorded in *T. parthenium* than in *T. vulgare* (df = 24, $t = 7.15$, $P < 0.0001$), and at higher EO concentrations (0.125 vs. 0.25%: df = 24, $t = 3.76$, $P = 0.0029$; 0.125 vs. 0.5%: df = 24, $t = 4.99$, $P = 0.0001$).

Our results also confirmed the phagodeterrent effects of *Tanacetum* oils on *L. decemlineata* adults. Similar to the results obtained in larvae, adult consumption differed significantly among the experimental groups after 96 h of exposure ($H_{6,35} = 22.11$, $P = 0.0012$), and the phagodeterrent effects strongly depended on the *Tanacetum* species (df = 24, $t = 6.82$, $P < 0.0001$) and EO concentration (0.125 vs. 0.25%: df = 24, $t = 5.59$, $P = 0.0029$; 0.125 vs. 0.5%: df = 24, $t = 7.84$, $P = 0.0001$) (table 2). It is clear that *Tanacetum* oils effectively reduced leaf consumption by adults. The values of leaf damage were significantly lower than in the control group, except in the group treated with the lowest concentration of *T. parthenium* EO (fig. 1).

The results presented in table 3 clearly demonstrate that leaf damage and antifeedant effects of *Tanacetum* oils were stage-specific. On average, during 96 h the adults consumed more leaves than the third instar larvae (fig. 1; significant developmental stage effect in table 3). Also, the EOs exhibited different effects depending on the developmental stage (significant $D \times EO$ interaction effect). *T. parthenium*

Table 1. Chemical composition of *T. parthenium* and *T. vulgare* EOs

Constituent	*T. parthenium* (%)	*T. vulgare* (%)
1,3-Cyclopentadiene-5-terc.butyl	1.30	–
*Tricyclene	0.41	0.07
*α-Pinene	1.51	0.42
*Camphene	8.07	0.37
*Sabinene	0.12	0.02
*β-Pinene	0.20	0.04
1,3,3-Trimethyl-bicyclo(2,2,2)oct-5-ene	–	0.04
α-Phellandrene	tr	–
α-Terpinene	0.07	–
*p-Cymene	0.98	1.02
1,8-Cineole	–	3.61
Limonene	0.43	–
*γ-Terpinene	0.22	0.05
β-Thujone	–	**49.75**
2,2,4-Trimethyl-3-cyclohexene-1-carbaldehyde	–	1.32
Thujyl alcohol	–	0.22
Linalool	0.53	–
*Camphor	**44.75**	8.20
*Borneol	0.34	1.24
*Terpinene-4-ol	0.33	0.39
*Myrtenal	0.17	0.18
α-Terpineol	–	0.08
exo-Borneol	–	0.28
*trans-Chrysanthenyl acetate	**28.97**	**22.27**
Carvotanacetone	–	tr
*Bornyl acetate	1.93	0.22
Myrtenyl acetate	0.09	–
Phenylmethylvalerate	tr	–
Trimethylphenylbutyrate	tr	–
α-Copaene	tr	–
trans-Caryophyllene	0.13	–
trans-β-farnesene	0.36	–
β-Cubebene	0.07	–
Bornyl valerate	tr	–
Bornyl angelate	0.59	–
α-Gurjunene	0.14	–
Carvacrol	–	0.12
β-Damascenone	–	0.05
β-Selinene	–	0.04
Spathulenol	–	0.31
Total	91.71	90.31
Monoterpene hydrocarbons	11.03	1.01
Oxygenated monoterpenes	77.70	87.93

(*Continued*)

Table 1. (*Continued.*)

Constituent	*T. parthenium* (%)	*T. vulgare* (%)
Sesquiterpene hydrocarbons	0.70	0.04
Oxygenated sesquiterpenes	–	0.31
Aromatic hydrocarbons	2.28	1.02

Major components (above 20%) are marked in bold. Components present in both oils are marked with an asterisk. Components present in traces are marked with tr.
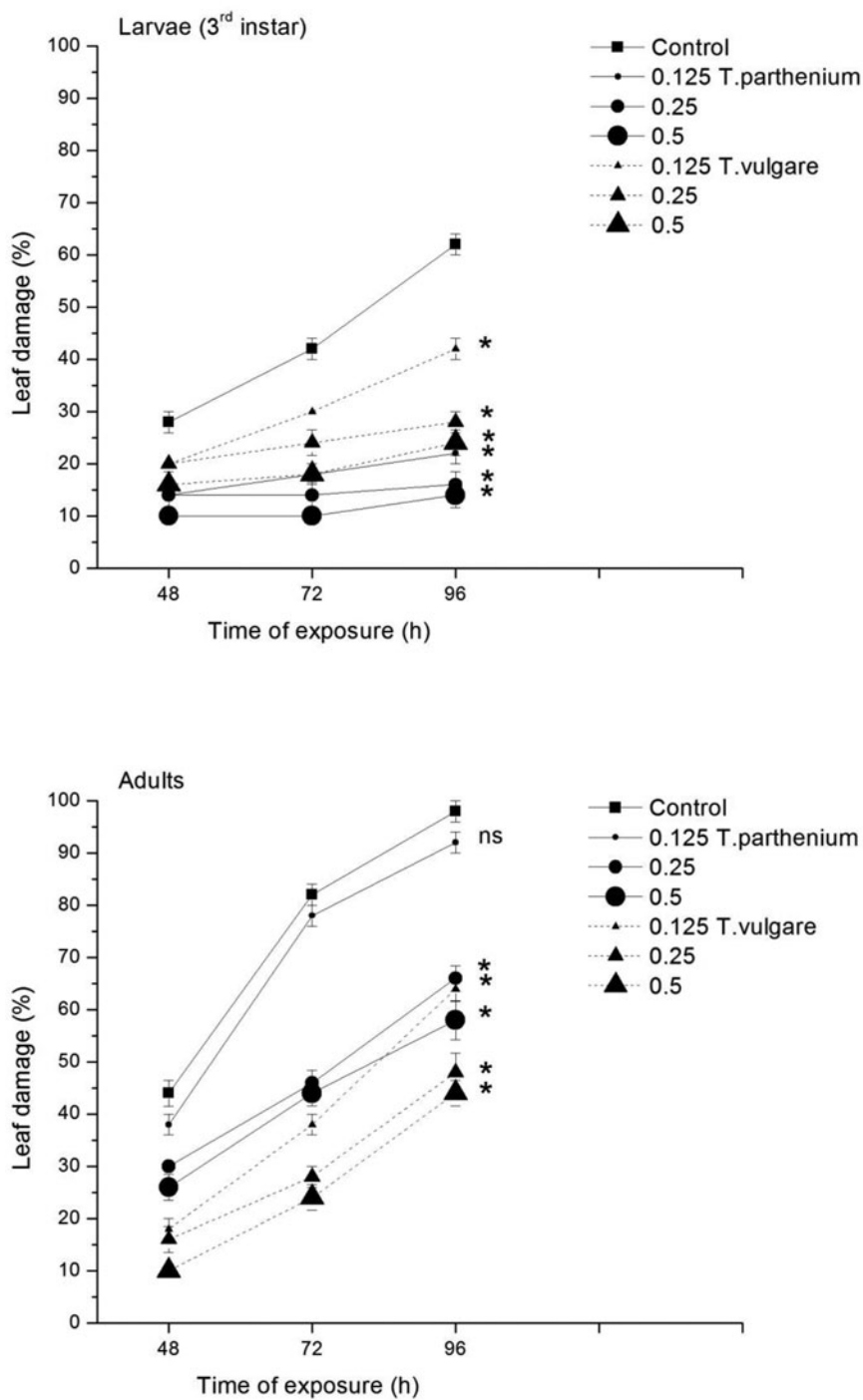

Figure 1. The percentage of the damage of potato leaves treated with different concentrations of *T. parthenium* and *T. vulgare* EOs after 2, 3 and 4 days of consumption by *L. decemlineata* third instar larvae and adults. Significant differences ($P < 0.05$) of treatment groups from the control in the level of leaf damage after 4 days are marked with an asterisk.

Table 2. Two-way non-parametric ANOVA testing effects of *Tanacetum* EOs, oil concentrations and their interaction at 96 h of leaf damage made by third instar larvae and adults of *L. decemlineata*

Source of variation	Third instar larvae			Adults		
	df	*F*	*P*	df	*F*	*P*
Essential oil (EO)	1, 19.9	51.11	**<0.0001**	1, 16.9	46.54	**<0.0001**
Concentration (*C*)	1.89, 19.9	12.41	**<0.0001**	1.87, 16.9	28.62	**<0.0001**
EO × *C*	1.89, 19.9	0.66	0.5071	1.87, 16.9	0.39	0.6656

Significant *P* values are presented in bold.

Table 3. Three-way non-parametric ANOVA testing effects of development stage, *Tanacetum* EOs, oil concentrations and their interaction at 96 h of leaf damage made by *L. decemlineata*

Source of variation	df	*F*	*P*
Dev. stage (*D*)	1, 38.7	572.30	**<0.0001**
Essential oil (EO)	1, 38.7	0.44	0.5064
Concentration (*C*)	1.91, 38.7	41.66	**<0.0001**
D × EO	1, 38.7	100.46	**<0.0001**
D × *C*	1.91, 38.7	0.55	0.5671
EO × *C*	1.91, 38.7	1.72	0. 1815
D × EO × *C*	1.91, 38.7	0.78	0.4539

Significant *P* values are presented in bold.

EO was more effective at reducing leaf damage by larvae, and *T. vulgare* EO was more effective in adults. The same pattern was also observed from the AFI (fig. 2). Another difference between developmental stages refers to the opposite trends of changes in the AFI with time. In larvae, antifeedant activity increased during the time of observation, while in adults it decreased.

Behavioural responses to Tanacetum *EO volatiles in the olfactometer*

Tanacetum EOs altered the responses of both developmental stages of *L. decemlineata* to host plant odour in the olfactometer in which contact cues with a stimulus were absent (table 4). The time needed to choose the control disc was prolonged when an alternative leaf disc was treated with the EO. On average, the two EOs did not differ in effectiveness (non-significant EO effect in table 5) while EO concentration significantly influenced movement of *L. decemlineata* larvae (0.125 vs. 0.5%: df = 24, t = 4.02, P = 0.0005) and adults (0.125 vs. 0.5%: df = 24, t = 3.41, P = 0.0037) (table 5). These patterns were similar in both the larvae and adults, i.e. the developmental stage ($F_{1,77.1}$ = 0.01, P = 0.9103) and D × EO effects were not significant ($F_{1,77.1}$ = 0.07, P = 0.7971). Larvae exposed to discs treated with the highest doses of both EOs, and adults exposed to the highest dose of *T. vulgare* EO moved more rapidly towards the control disc and were as fast as individuals that were presented with two control discs (table 4).

Behavioural responses to Tanacetum *EO volatiles after contact with a stimulus*

After direct contact with *Tanacetum* EOs, *L. decemlineata* larvae rapidly moved away from the stimulus. On average, such a
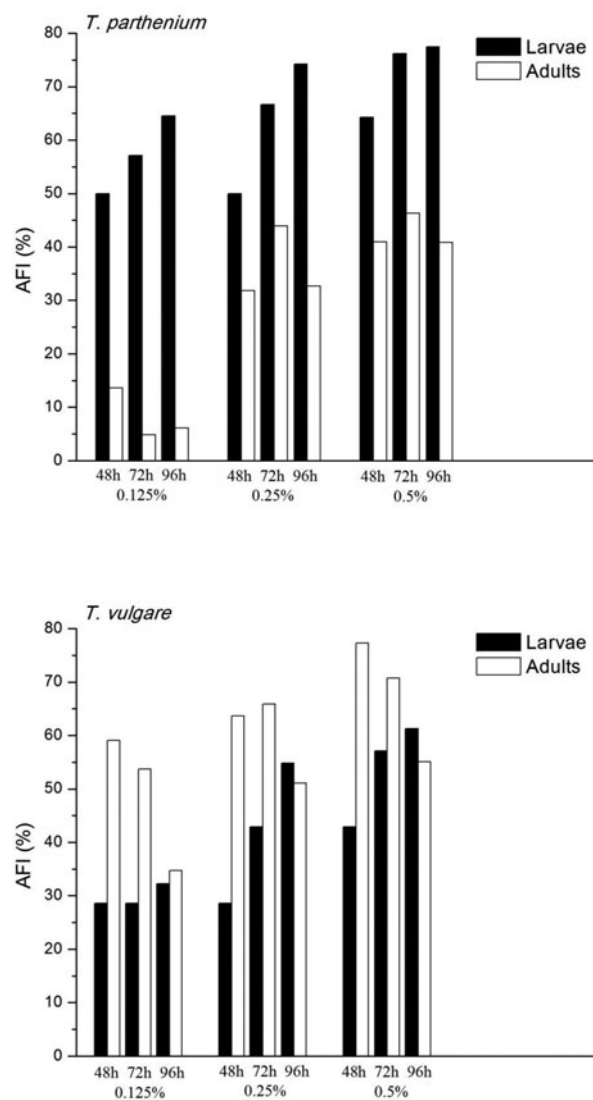

Figure 2. AFI for *L. decemlineata* larvae and adults after 2, 3 and 4 days of consumption of potato leaves treated with *T. parthenium* and *T. vulgare* EOs at three different concentrations (0.125, 0.25 and 0.5%).

behavioural response was provoked more rapidly by *T. parthenium* than by *T. vulgare* EO (table 4; significant EO effect in table 5), and by higher than lower EO concentrations (0.125 vs. 0.5%: df = 24, t = 5.91, P < 0.0001; 0.25 vs. 0.5%: df = 24, t = 4.46, P = 0.0001) (table 4; significant concentration effect in table 5). The effect of the concentration of EO depended on the

Table 4. Behavioural responses of *L. decemlineata* to *T. parthenium* and *T. vulgare* EOs in non-contact (choice time in s) and contact (escape time in s) bioassays

	Conc.	Choice time – larvae		Choice time – adults		Escape time – larvae	
		X	±SE	*X*	±SE	*X*	±SE
T. parthenium	0.125	**220.0**	**9.94**	**276.3**	**45.51**	120.2	14.97
	0.25	**183.5**	**20.51**	**183.9**	**35.03**	97.6	16.43
	0.5	153.6	32.23	**174.1**	**35.11**	23.0	3.19
T. vulgare	0.125	**210.1**	**16.27**	**233.4**	**22.80**	115.1	14.26
	0.25	**162.2**	**23.34**	163.1	45.09	97.2	17.94
	0.5	108.5	15.45	105.6	14.16	85.8	15.46
Control	0	70.90	10.75	72.90	14.08		
ANOVA		$F_{6,27.61} = 18.82$, $P < 0.0001$		$F_{6,63} = 5.21$, $P = 0.0002$		$F_{5,54} = 12.96$, $P < 0.0001$	

F, *P* values – results of Welch's one-way ANOVA for larval choice time and results of classic one-way ANOVA for adult choice time and larval escape time. Significant differences ($P < 0.05$) from the control group are marked in bold.

Table 5. Results of two-way ANOVA testing of the effects of *Tanacetum* oils, EO concentrations and their interaction on larval choice time (non-parametric ANOVA), adult choice time and larval escape time (classic ANOVA)

Source of variation	Larval choice time			Adult choice time			Larval escape time		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Essential oil (EO)	1, 37.5	2.51	0.1132	1, 54	1.67	0.2021	1, 54	7.26	**0.0094**
Concentration (*C*)	1.9, 37.5	7.67	**0.0006**	2, 54	6.20	**0.0038**	2, 54	18.96	**<0.0001**
EO × *C*	1.9, 37.5	0.43	0.6428	2, 54	0.23	0.7971	2, 54	9.80	**0.0002**

Significant effects are marked in bold.

Discussion

Tanacetum species (significant EO × *C* interaction in table 5). It can be seen in table 4 that a significant decrease in escape time with an increasing concentration of EO was observed only in *T. parthenium* oil (0.125 vs. 0.5: df = 54, *t* = 6.87, *P* < 0.0001).

Our results showed that the two examined *Tanacetum* oils produced repellent effects on *L. decemlineata*. Treatment with the EOs reduced potato leaf damage, slowed down movement towards the untreated leaf disc in the olfactometer and increased movement away from the treated leaf disc. The following patterns were revealed: (1) The efficacy of *Tanacetum* EOs in modifying *L. decemlineata* behaviour was developmental-stage-specific and depended on *Tanacetum* species and EO concentration. (2) Sensitivity of leaf damage and choice time to increasing concentrations of EOs did not differ significantly between *Tanacetum* species and developmental stages. (3) *T. parthenium* and *T. vulgare* EOs were not equally effective at reducing leaf damage by larvae and adults.

Many studies have shown antifeeding and avoidance behaviours of *L. decemlineata* in response to different plant extracts and EOs (González-Coloma *et al.*, 2006; Pavela, 2010; Gökçe *et al.*, 2012; Bekircan *et al.*, 2015). These types of behaviours are induced by compounds that hinder recognition of host-indicating odours and thus host acceptance for oviposition and feeding (Schröder and Hilker, 2008). Panasiuk (1984) recorded movement of *L. decemlineata* adults away from filter paper treated with a single concentration of thujone-rich

T. vulgare EO, and also described adult behaviour in short-term choice tests as avoidance, i.e. beetles exclusively chose untreated leaves and fed on them. This is in accordance with our previous finding that *L. decemlineata* adults never choose leaves treated with thujone-rich *T. vulgare* EO (Kostić *et al.*, 2003). In contrast, a repellence index below 100% was recorded with EOs from bornyl acetate and camphor-umbellulone *T. vulgare* chemotypes (Schearer, 1984). Herein, we used choice time to quantify the repellent behaviour of *L. decemlineata* in an olfactometer choice test and found that both the larvae and adults respond to EO treatment by choice time prolongation in a dose-dependent manner.

When there was no choice, within the first minutes of the contact, *L. decemlineata* rapidly moved away from the treated leaves. However, after 2–4 days, potato leaves treated with *Tanacetum* oils were consumed although at a lower rate than the control potato leaves. Again, these behavioural responses depended on EO concentration. Similarly, a dose-dependent antifeedant activity in *L. decemlineata* was recorded for water and organic solvent extracts of *T. vulgare* (Hough-Goldstein, 1990; Kutas and Nádasy, 2005) and other plant species (Gökçe *et al.*, 2012; Alkan *et al.*, 2015; Rusin *et al.*, 2016), and for the EOs of *Satureja hortensis*, *Thymus transcaucasicus*, *T. pseudopulegioides* and *T. leucotrichus* (Pavela *et al.*, 2009; Bekircan *et al.*, 2015). Antifeedant activity of EOs was achieved at lower doses when compared to water and methanolic extracts, which can be explained by differences in composition and the concentrations of active ingredients. Comparing our results on the AFI obtained after 3 days of *L. decemlineata* exposure to 0.5% concentration of *Tanacetum* EOs

with the results of other authors revealed that *Tanacetum* EOs were stronger antifeedants than *T. leucotrichus* EO and the methanolic extract of *Humulus lupulus* (Gökçe *et al.*, 2012; Bekircan *et al.*, 2015), while the hexane-ethylacetate-methanolic extracts of *Achillea millefolium* and *H. lupulus* induced similar antifeedant response (Alkan *et al.*, 2015).

Our results obtained in behavioural assays, which include direct contact between *L. decemlineata* and EOs show that the repellent responses differed between *T. vulgare* and *T. parthenium*. Namely, the antifeedant bioassay revealed that the *T. parthenium* EO was more effective at reducing leaf damage by larvae, and that the *T. vulgare* EO was more effective in adults. Also, the *T. parthenium* EO produced more rapid movement away from the treated leaf disc than *T. vulgare*. Differences in oil composition may account for significant differences in efficacy between the two *Tanacetum* oils.

With regards to a major terpene, more than 30 different chemotypes can be distinguished in *T. vulgare*, while *T. parthenium* was less diverse (Kleine and Müller, 2011). β-Thujone-chrysanthenyl acetate chemotype of *T. vulgare* in the current paper, is similar to samples from Estonia (Raal *et al.*, 2014), and the camphorchrysanthenyl acetate *T. parthenium* chemotype corresponds to individuals collected in Belgium (De Pooter *et al.*, 1989) and the Netherlands (Hendriks *et al.*, 1996). Compounds such as camphor, β-thujone, camphene, bornyl acetate, α-pinene, 1,8-cineole, *p*-cymene that are known for repellent effects against *L. decemlineata* (Panasiuk, 1984; Schearer, 1984), are present at concentrations above 1% at least in one of the two examined *Tanacetum* EOs. Dominant components of the EOs belong to oxygenated monoterpenes, which are thought to contribute to antifeedant activity in insects (Koul, 2004). Kostić *et al.* (2007) recorded antifeedant effects of the thujone-rich fraction of *Salvia officinalis* EO, and of camphor in larvae and adults of *L. decemlineata*. The higher percentage of oxygenated monoterpenes in the *T. vulgare* EO than in the *T. parthenium* EO possibly contributed to its higher efficacy in adults.

Interestingly, opposite results were obtained for larvae, so that the *T. parthenium* EO was more effective at reducing feeding than the EO of *T. vulgare*. It has been suggested by Hough-Goldstein (1990) that adults, as primary host finders, are more sensitive to deterrents than larvae. However, our results, as well as the results of other authors (Szczepanik *et al.*, 2005; Kutas *et al.*, 2009; Rusin *et al.*, 2016), revealed that larva-adult differences in repellent behaviour depended on the botanical type and concentration.

Chemical insecticides are effective in potato protection as they cause high mortality of *L. decemlineata* larvae and adults, and significantly reduce leaf damage in the field (Alyokhin *et al.*, 2007; Bassi *et al.*, 2009). However, eco-friendly botanical insecticides such as neem extracts, pyrethrin and stilbenes could be equally or more successful, inducing 2.5–8-fold lower leaf damage (Igrc Barčić *et al.*, 2006; Gabaston *et al.*, 2018). Our laboratory assays showed that after 3 days of exposure to the highest applied concentrations of EOs, leaf damage was about fourfold lower in *T. parthenium* EO in larvae, and in *T. vulgare* EO in adults. The antifeedant effects of *Tanacetum* oils were apparently stronger than the effects obtained in laboratory assays after 3 days of exposure of *L. decemlineata* to sublethal concentrations of the chemical insecticides imidacloprid and cyromazine (Furlong and Groden, 2001). On the other hand, a 3-day assay with neembased botanical insecticides showed that *Tanacetum* EOs were more effective for adults than for larvae (Zehnder and Warthen, 1988; Hiiesaar *et al.*, 2009).

In conclusion, these results point to the potential for the development of a *Tanacetum* EO-based repellent that could be included in integrated pest management programmes against *L. decemlineata*. Such a repellent would not be toxic to humans since it is known that the acceptable daily intake of camphor and thujone is relatively high (Zuccarini, 2009; Lachenmeier and Uebelacker, 2010). However, before its commercial application, several issues should be addressed, such as risks for nontarget organisms, provision of increased EO stability, water solubility and persistence in the environment, as well as efficacy against the target *L. decemlineata* under field conditions.

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