

**EFFECTS OF AROMATIC PLANT EXTRACTS
AND MAJOR TERPENOID CONSTITUENTS
ON FEEDING ACTIVITY OF THE HORSE-CHESTNUT
LEAF MINER *CAMERARIA OHRIDELLA*
DESCHKA & DIMIĆ 1986***

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Abstract

Chemical control of the horse-chestnut leaf miner *Cameraria ohridella* is limited to microinjections that injure trees, for air pesticide spraying is not recommended in urban areas. Alternative methods, e.g., the use of antifeedants to control and/or prevent the *C. ohridella* infestations are searched for. The aim of the present study was to assess the effect of selected aromatic plant extracts and their major terpenoid constituents on the feeding of *C. ohridella* larvae. We found that an extract of *Solidago canadensis* was attractant, the extracts of *Tanacetum vulgare* and *Heracleum mantegazzianum* were potentially attractant, *Pimpinella anisum*, *Carum carvi*, *Syzygium aromaticum*, *Thuja occidentalis*, *Origanum majorana* were inactive, and *Thymus vulgaris*, *Satureja hortensis*, *Rosmarinus officinalis* were potentially deterrent. β -Pinene, geraniol, linalool, and *p*-cymene were attractant, γ -terpinene and linalool were potentially attractant, *h*-terpinene, terpinolene, and camphene were inactive, bornyl acetate and α -pinene – potentially deterrent, and thymol was deterrent. Complex plant extracts against *C. ohridella* larvae are less active than pure compounds. The horse chestnut characteristic saponin β -aescin appeared a feeding stimulant for *C. ohridella*.

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WPLYW OLEJKÓW ETERYCZNYCH ROŚLIN AROMATYCZNYCH
ORAZ ICH NAJWAŻNIEJSZYCH SKŁADNIKÓW TERPENOIDOWYCH NA ŻEROWANIE
SZROTÓWKA KASZTANOWCOWIACZKA *CAMERARIA OHRIDELLA*
DESCHKA & DIMIĆ 1986

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Słowa kluczowe: antyfidanty, stymulacja żerowania, β -escyna, tymol, *Cameraria ohridella*.

Abstrakt

Chemiczne zwalczanie szrotówka kasztanowcowiaczka *Cameraria ohridella* jest ograniczone do mikroiniekcji, które mogą powodować uszkodzenia drzew. Stosowanie opryskiwania nie jest polecane w terenach zurbanizowanych. Poszukiwane są alternatywne metody zwalczania szrotówka lub przeciwdziałania zasiedlaniu drzew przez tego owada np. wykorzystanie antyfidantów. Celem tej pracy było zbadanie wpływu olejków eterycznych roślin aromatycznych oraz ich najważniejszych składników terpenoidowych na żerowanie *C. ohridella*. Stwierdzono, że ekstrakt z *Solidago canadensis* miał właściwości przywabiające, ekstrakty z *Tanacetum vulgare* i *Heracleum mantegazzianum* potencjalnie przywabiające, *Pimpinella anisum*, *Carum carvi*, *Syzygium aromaticum*, *Thuja occidentalis*, *Origanum majorana* były nieaktywne, a ekstrakty z *Thymus vulgaris*, *Satureja hortensis*, *Rosmarinus officinalis* wykazywały właściwości potencjalnie deterentne. Spośród terpenoidów β -pinen, geraniol, linalool, i *p*-cymen miały właściwości przywabiające, γ -terpinen i linalool potencjalnie przywabiające, α -terpinen, terpinolen i kamfen nie miały wpływu na zachowanie larw szrotówka, a octan bornylu i α -pinen wykazywały działanie potencjalnie deterentne, natomiast tymol – działanie deterentne. β -escyna, saponina charakterystyczna dla kasztanowca zwyczajnego, stymulowała żerowanie larw szrotówka kasztanowcowiaczka.

Introduction

The horse-chestnut leaf miner (*Cameraria ohridella* Deschka & Dimić 1986) (Lepidoptera: Gracillariidae) appeared in Macedonia in 1985 but the place of its origin is still unknown (TOMICZEK and KREHAN 1998). In Poland, it was recorded for the first time in Wojsławice near Wrocław, south-west Poland, in 1998 (ŁABANOWSKI, SOIKA 1998, BARANIAK et al. 2004, AUGUSTIN et al. 2009). In Poland, *C. ohridella* develops usually three generations a year and the mining larvae feed between the two epidermis layers, which may cause the defoliation of trees already in summer (SUKOVATA et al. 2011).

Generally, the horse-chestnut leaf miner is defined as a monophagous species almost exclusively developing on white-blooming horse chestnut trees

Aesculus hippocastanum L. *C. ohridella* is occasionally found on other *Aesculus* species, such as Japanese horse chestnut *A. turbinata* Blume, yellow buckeye *A. flava* Sol., or red buckeye *A. pavia* L., and also *Acer platanoides* L. and *A. pseudoplatanus* L. adjacent to heavily infested horse-chestnut trees (AUGUSTIN et al. 2009). The red horse-chestnut *A. x carnea* Hayne is rarely considered a host of *C. ohridella* because despite the abundant oviposition by females of each generation, the larvae usually die within leaf tissues before they reach the third stage (KUKUŁA-MŁYNARCZYK et al. 2006). However, DZIĘGIELEWSKA and KAUP (2007) found that the horse-chestnut leaf miner is able to develop two full generations on the red horse-chestnut under special circumstances: heavy infestation of white horse chestnut in the vicinity, high *C. ohridella* population numbers and mild winters in several consecutive years at the location. Nevertheless, the number of larvae and the leaf damage on the red horse-chestnut are relatively low, i.e. maximum 10% of the leaf has been damaged throughout the vegetative period (DZIĘGIELEWSKA and KAUP 2007).

Considering the rapid dispersal, the mass appearance, and high biological potential of the horse-chestnut leaf miner as well as the fact that the horse chestnut is one of the most abundant ornamental trees in parks and other public areas in Europe, many methods have been proposed to control the pest: biological, cultural, and chemical. The natural biological control of *C. ohridella* is limited due to a narrow spectrum and low impact of natural enemies: the parasitism level is below 10% and the predation rates of birds, mainly the tits, range from 2 to 4% of leafminer populations (GRABENWERGER 2003, GRABENWERGER et al. 2005). Cultural control by removing fallen leaves of horse chestnut in autumn contributes significantly to reduce *C. ohridella* damage and it is suggested for application in the context of Integrated Pest Management (PAVAN et al. 2003, BARANIAK et al. 2004). However, this kind of control measure is difficult to apply on a large scale, so it is recommended rather in small and isolated stands of the horse-chestnut trees (PAVAN et al. 2003). Chemical control can be applied either by tree injection or aerial spraying. Tree injections are expensive and the holes drilled into the trunk can injure trees (TOMCZYK et al. 2007). Aerial spraying has limited use due to environmental concerns: broad spectrum insecticides are proposed and the spraying involves urban areas usually densely inhabited by people (KOBZA et al. 2011). Considering these limitations, alternative methods of control and/or prevention of *C. ohridella* infestations are studied. For example, attempts to control *C. ohridella* using an attract-and-kill technique have been made: the formulation of *C. ohridella* sex pheromone and a fast acting contact toxicant was applied directly to the bark of the trees but no effect on leaf infestation was found (SUKOVATA et al. 2011). Another method studied is to disrupt host recognition behaviour, affect the oviposition, and/or deter the feeding

of *C. ohridella* by applying plant-derived (botanical) insecticides or deterrents. For example, the application of plant allelochemicals to the horse chestnut foliage may alter the behavioural response of adult *C. ohridella*: the extract of red chestnut leaves sprayed on white horse chestnut leaves decreased the attractiveness of sprayed areas to the adults of *C. ohridella* (TOMCZYK et al. 2008). Extract of *Polyscias filicifolia* Bailey (Araliaceae) was repellent to the females of the horse chestnut leaf miner, which resulted in the lower number of eggs laid on treated leaves as compared to the control (TOMCZYK et al. 2007).

The aim of the present study was to evaluate the effect of essential oils of common aromatic and medicinal plants on the feeding activity of *C. ohridella* larvae. Moreover, we examined the response of the larvae to major terpenoid ingredients of the most active essential oils in this study and to β -aescin, which is the main biologically active saponin constituent of horse chestnut (KALEMBA et al. 2001, SIRTORI 2001, SEDLAKOVA et al. 2003, TOUAFEK et al. 2004, BHUIYAN et al. 2010, TKACHENKO 2010, MAHBOUBI, KAZEMPOUR 2011, SZCZEPANIK, SZUMNY 2011, TSAI et al. 2011, RAINA, NEGI 2012, SZCZEPANIK et al. 2012). Essential oils of common aromatic plants are known for their broad spectrum of biological activity: antiseptic (i.e., bactericidal, virucidal and fungicidal), and medicinal (e.g., antioxidant and anticancer) (BAKKALI et al. 2008, ZU et al. 2010, HUSSAIN et al. 2011). Aromatic plants and essential oils have also been applied for the protection of food, plants, and animals against pests and diseases (PICKETT et al. 1997, ISMAN 2000, BAKKALI et al. 2008,) because they are generally nontoxic to mammals, birds, and fish (KOUL et al. 2008). The extracts of the following material was used: dried seeds of caraway *Carum carvi* L., giant hogweed *Heracleum mantegazzianum* Sommier & Levier, fruit of anise *Pimpinella anisum* L., dried buds of cloves *Syzygium aromaticum* (L.) Merrill & Perry, fresh green parts of marjoram *Origanum majorana* L., oregano *O. vulgare* L., rosemary *Rosmarinus officinalis* L., thyme *Thymus vulgaris* L., goldenrod *Solidago canadensis* L., tansy *Tanacetum vulgare* L., leaves and seeds of *Thuja occidentalis* L., and dried summer savory *Satureja hortensis* L.

Material and Methods

Plant extracts and pure compounds

The plant material was obtained from Svedeponic Company (Kraśnicza Wola) and from Kawon-Hurt (Krajewice). Essential oils were obtained from plant material by hydrodistillation on Deryng apparatus (SZUMNY et al. 2010). In brief, approximately 200 g of fresh or 50 g of dried plant material was placed

in a 2 L round flask together with 900 mL of distilled water. The sample flask was heated for 2 h after the boiling point was reached. The vapors were condensed by means of a cold refrigerant. After 120 min of distillation, depending on plant material, from 0.25 to 2.5 mL of essential oil containing the volatile compounds was collected in a 2.5 mL vial and kept at -15°C until the GC-MS analyses and biological tests were performed. The identification and quantification of the volatile compounds was performed using a gas chromatograph (GC) coupled to a mass spectrometer (MS), a Saturn 2000 MS Varian Chrompack with a DB-5. Most of the compounds were identified by using three different analytical methods: (1) Kovats indices, (2) GC-MS retention indices with authentic chemicals – standards and (3) mass spectra (authentic chemicals and NIST05 spectral library collection (MS). Basing on the results of chemical analysis (SZCZEPANIK and SZUMNY 2011, SZCZEPANIK et al. 2012), the following major terpenoid components of the extracts studied were bioassayed: bornyl acetate, camphene, p-cymene, geraniol, linalool, α -pinene, β -pinene, α -terpinene, γ -terpinene, terpinolene, and thymol. All pure chemicals were purchased from Sigma-Aldrich.

Biological assays

White horse chestnut (*A. hippocastanum*) compound leaves with visible mines of the first generation larvae of the horse chestnut leaf miner (*C. ohridella*) were collected and transferred to laboratory. The mines were examined for the presence of larvae using a binocular microscope and the t_0 (=time of application) area of the mine was calculated according to a formula: $A_0 = \pi ab$, assuming the elliptical shape of the mine (A_0 – area of the ellipse at t_0 ; a and b – one-half of the ellipse's major and minor axes, respectively). 15 μL of the studied substances (1% ethanolic solutions; 20 replications per studied substance) were applied to the surface of the mine using a BRAND Transferpette[®]. The leaves were put individually in flasks containing water and placed in the growing chamber Sanyo ($t = 23^{\circ}\text{C}$, L16 : D8) for five days. After the incubation period, the t_1 (=time after incubation period) area of the mines was calculated, according to a formula: $A_1 = \pi a_1 b_1$. The feeding activity index (FAI) of individual larvae was determined using a formula: $\text{FAI} = A_1/A_0$. The data thus obtained were log transformed and analysed statistically using two-tailed Dunnett's test to find significant differences between individual treatments and control (untreated mines) at $p < 0.05$.

Results and Discussion

The essential oils from aromatic plants and pure compounds applied in the present study had a varied effect on the feeding of *C. ohridella* larvae. The essential oil from *S. canadensis* caused statistically significant increase in the feeding activity of the horse chestnut leaf miner larvae: a 1.6-fold increase in plant tissue consumption by *C. ohridella* was found after the application of this oil (Table 1). The effects of *T. vulgare* and *H. mantegazzianum* oils were also relatively strong (1.3- and 1.2-fold increase in tissue consumption, respectively) but the effect was not statistically significant.

Table 1
Feeding Activity Indices (FAI) of the horse chestnut leaf miner *Cameraria ohridella* larvae after application of essential oils from aromatic plants^a

Plant essential oil	FAI ^b	<i>p</i> ^c
Control	1.94 (+1.23)	
<i>Carum carvi</i>	1.86 (±0.82)	0.997
<i>Haracleum mantegazzianum</i>	2.42 (±0.99)	0.136
<i>Origanum majorana</i>	1.96 (±2.38)	0.587
<i>Origanum vulgare</i>	2.13 (±1.53)	0.700
<i>Pimpinella anisum</i>	2.33 (±0.95)	0.211
<i>Rosmarinus officinalis</i>	1.42 (±0.62)	0.187
<i>Satureja hortensis</i>	1.60 (±1.02)	0.329
<i>Solidago canadensis</i>	2.99 (±2.18)	0.026
<i>Syzygium aromaticum</i>	2.21 (±1.21)	0.433
<i>Tanaceum vulgare</i>	2.55 (±2.01)	0.243
<i>Thuja occidentalis</i> leaves	2.04 (±1.18)	0.755
<i>Thuja occidentalis</i> seeds	2.31 (±1.44)	0.373
<i>Thymus vulgaris</i>	1.69 (±1.17)	0.458

^a Numbers are the means of 20 replicates and are presented with standard deviations (±SE),

^b FAI – Feeding Activity Index, ^c Two-tailed Dunnett's test was applied to find significant differences between individual treatments and control at *p*<0.05

The horse chestnut characteristic saponin β -aescin appeared a feeding stimulant for *C. ohridella*: 1.7-fold increase in plant tissue consumption occurred after the application of this compound (Table 2). A significant increase in the feeding activity of larvae occurred after the application of β -pinene, geraniol, linalool, and *p*-cymene (1.7, 1.6, 1.5, and 1.5-fold increase, respectively). The application of thymol caused a significant decrease in the feeding activity of larvae (nearly 60% less consumed food as compared to control) – Table 2.

Table 2
Feeding Activity Indices (FAI) of the horse chestnut leaf miner *Cameraria ohridella* larvae after application of individual terpenoids and β -aescin^a

Terpenoid	FAI ^b	p ^c
Control	1.94 (\pm 1.23)	
β -Aescin	3.32 (\pm 1.75)	0.002
Bornyl acetate	1.74 (\pm 0.91)	0.681
Camphene	1.99 (\pm 0.87)	0.694
p-Cymene	2.87 (\pm 1.46)	0.026
Geraniol	3.01 (\pm 2.12)	0.011
(\pm)-Linalool	2.38 (\pm 1.88)	0.476
(-)-Linalool	2.9 (\pm 1.38)	0.016
α -Pinene	1.36 (\pm 0.46)	0.157
β -Pinene	3.38 (\pm 3.19)	0.013
Terpinolene	2.01 (\pm 1.19)	0.804
α -Terpinene	2.06 (\pm 1.77)	0.967
γ -Terpinene	2.49 (\pm 1.04)	0.105
Thymol	1.08 (\pm 0.21)	0.018

^a Numbers are the means of 20 replicates and are presented with standard deviations (\pm SE),

^b FAI – Feeding Activity Index, ^c Two-tailed Dunnett's test was applied to find significant differences between individual treatments and control at $p < 0.05$

The total and relative content of individual components in plant tissues depends on many factors, such as the botanical variety, vegetative stage, plant part, time of harvest, geographical location, e.t.c (WITTSTOCK, GERSHENZON 2002). However, the composition of the set of allelochemicals in aromatic plants is species- or at least genus-specific. Nevertheless, it must be kept in mind that plants usually contain more than one group of protective anti-herbivore secondary metabolites (GABRYŚ, PAWLUK 1999) This particular attribute may be responsible for *C. ohridella* response to some of the essential oils applied in this study. Interestingly, the goldenrod extract was highly attractive to *C. ohridella* larvae despite the content of α -pinene and bornyl acetate, which showed potentially deterrent activity when applied individually. Goldenrods, the plants of the genus *Solidago*, contain considerable amount of flavonoids and saponins that are responsible for many biological (e.g., therapeutic) effects of these plants (KOŁODZIEJ et al. 2011). The highly attractive to *C. ohridella* β -aescin is a saponin, so it is possible that the weakly deterrent effect of *Solidago* terpenoids was 'overshadowed' by attractant properties of saponins. However, the presence of saponins in the essential oil studied remains to be confirmed in the further study. The lower potency of attractant properties of tansy and giant hogweed extracts to *C. ohridella* larvae is

probably caused by the complexity of terpenoid composition in their essential oils. On one hand, tansy contains highly attractive β -pinene and p-cymene but on the other hand – the deterrent α -pinene is present as well (WOLF et al. 2012). Likewise, giant hogweed contains the attractive β -pinene and γ -terpinene, which may be 'screened' by the deterrent α -pinene (TKACHENKO 2010). Similarly, the highly deterrent effect of thymol may be neutralized by geraniol, linalool and p-cymene in the thyme extract (GRIGORE et al. 2010, ZU et al. 2010, SZCZEPANIK et al. 2012), and by p-cymene in the summer savory extract (MAHBOUBI, KAZEMPOUR 2011). The rosemary oil has probably the highest feeding deterrent potential to *C. ohridella*: its oil contains considerable amounts of α -pinene and bornyl acetate (PINTORE et al. 2009) and in present experiments it caused the highest reduction in food consumption by *C. ohridella* larvae.

In conclusion, the extracts studied can be divided into four groups according to the effect they had on *C. ohridella* larvae: attractant (goldenrod), potentially attractant (tansy and giant hogweed), inactive (anise, thuja, clove, oregano, marjoram, caraway), and potentially deterrent (thyme, summer savory, rosemary). The individual terpenoids can be divided into five groups: attractant (β -pinene, geraniol, (-)-linalool, p-cymene), potentially attractant (γ -terpinene, (+/-)-linalool), inactive (α -terpinene, terpinolene, camphene), potentially deterrent (bornyl acetate, α -pinene), and deterrent (thymol). Thymol, due to its feeding deterrent activity can be considered in the Integrated Pest Management (IPM) programmes against *C. ohridella* larvae, probably as a supplementary behaviour-controlling allelochemical.

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