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FEEDING DETERRENT ACTIVITY OF NATURAL MONOTERPENOIDS AGAINST LARVAE OF THE LARGE WHITE BUTTERFLY *PIERIS BRASSICAE* (L.)

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Key words: antifeedants, *Pieris brassicae*, monoterpenoids, large white butterfly, plant protection.

Abstract

The large white butterfly *Pieris brassicae* (L.) (Lepidoptera: Pieridae) is a cosmopolitan insect that is specialized to feed on the plant family Brassicaceae. The caterpillars may cause severe losses in yield of cabbage plants. In search of environmentally safe control chemical substances, the effect of 17 natural monoterpenes on the feeding activity and food assimilation of *P. brassicae* larvae was studied. According to the analysis of behavioural and physiological effects caused by the substances applied in the present study, these chemicals can be divided into into five groups: highly active deterrents that practically completely inhibited the feeding of caterpillars (α-phellandrene and β-ionone), strong deterrents (α-terpinene and α-ionone), relatively strong deterrents (citronellol, (-)-linalool, *p*-cymene), moderate deterrents ((+)-fenchone, (+)-R-limonene, γ-terpinene, and (S)-(+)-carvone), and inactive substances (α-pinene, eucalyptol, bornyl acetate, geraniol, thymol, and L-menthol). α-Phellandrene, β-ionone, α-ionone, α-terpinene, citronellol, and (-)-linalool may be classified as preingestive deterrents that act before food ingestion. *p*-Cymene may be considered as postingestive deterrent that causes a loss in body weight.

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AKTYWNOŚĆ DETERENTNA NATURALNYCH MONOTERPENOIDÓW WOBEC GĄSIENIC BIELINKA KAPUSTNIKA *PIERIS BRASSICAE* (L.)

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Słowa kluczowe: antyfidanty, *Pieris brassicae*, monoterpenoidy, bielinek kapustnik, ochrona roślin.

Abstrakt

Bielinek kapustnik *Pieris brassicae* (L.) (Lepidoptera: Pieridae) jest kosmopolitycznym gatunkiem wyspecjalizowanym w żerowaniu na roślinach z rodziny Brassicaceae. Gąsienice mogą powodować poważne straty u roślin kapustowatych. W poszukiwaniu przyjaznych środowisku środków ochrony roślin zbadano wpływ 17 naturalnych monoterpenoidów na żerowanie i przyrost masy ciała gąsienic *P. brassicae*. Analiza behawioralnych i fizjologicznych efektów wywołanych przez poszczególne substancje pozwoliła na zaliczenie ich do pięciu grup: bardzo aktywne deterenty, które prawie całkowicie zniechęcały gąsienice do żerowania (α-felandren i β-jonon), silne deterenty (α-terpinen i α-jonon), stosunkowo silne deterenty (citronellol, (-)-linalool, p-cymen), słabe deterenty ((+)-fenchon, (+)-R-limonen, γ-terpinen i (S)-(+)-karwon) oraz substancje nieaktywne (α-pinen, eukaliptol, octan bornylu, geraniol, tymol i L-mentol). Ponadto najbardziej aktywne deterenty (α-felandren, β-jonon, α-jonon, α-terpinen, citronellol, (-)-linalool) można określić jako deterenty smakowe działające przed pobraniem pokarmu, zaś *p*-cymen – jako deterent metaboliczny działający po pobraniu pokarmu i powodujący spadek masy ciała gąsienic.

Introduction

The large white butterfly *Pieris brassicae* (L.) (Lepidoptera: Pieridae) is specialized to feed on the plant family Brassicaceae. It is a cosmopolitan insect species in Europe and it occurs wherever cruciferous plants are grown. The larvae of *P. brassicae* feed on cabbage foliage and create large, irregular holes. In consequence, the severe reduction of the marketable yield or complete destruction of the crop occurs (JANKOWSKA 2006, JANKOWSKA et al. 2009, 2011). Till now, the control of *P. brassicae* has been oriented towards the use of efficient but high-risk insecticides. Considering various negative effects of their application there is an increasing demand for more specific, indirectly acting crop protection agents, such as repellents, insect-growth regulators, oviposition inhibitors, and antifeedants, which might, at least in part, replace conventional insecticides (SCHOONHOVEN 1982, VAN BEEK, DE GROOT 1986, LAY, TOOGOOD 1990, NORIN 2007). Of the behaviour-controlling chemicals, insect antifeedants have attracted a lot of research in the recent years, and the most interesting discoveries included the terpenoids of plant origin: ajugarin, azadirachtin, and polygodial. An antifeedant (= feeding deterrent) is a behaviour modifying substance that deters feeding through a direct action on peripheral sensilla (= taste organs) of insects (ISMAN 2002). The most spectacular antifeedant effects on *P. brassicae* larvae were reported by SHARMA and GUPTA (2009), who showed that the aqueous extract of *Azadirachta indica* and *Melia azedarach* protected 94.0 and 89.2 percent cabbage foliage against *P. brassicae*, respectively. Similar results were obtained by WAWRZYNIAK and WRZESIŃSKA (2000). Moreover, synergistic effect of these botanicals on the virulence of granulosis virus (GV) against *P. brassicae* was also found (BHANDARI et al. 2009). A number of terpene-containing plants that are native to Polish flora have also been studied in respect of feeding deterrent activity to *P. brassicae*. For example, WAWRZYNIAK (1996), studied the activity of aqueous as well as alcohol extracts of 65 plant species of 21 families, both in the field and in the laboratory. The results demonstrated a high feeding and oviposition deterrent activity of *Ajuga reptans*, *Callendula officinalis* and of the plants of Apiaceae and Geraniaceae families. The individual terpenoid constituents of aromatic plants were highly deterrent to other insect species, e.g. peach potato aphid *Myzus persicae* (GABRYŚ et al. 2005).

The aim of the present work was to assess the behavioural and physiological responses of the large white butterfly larvae to selected lower plant terpenoids. The effect of 17 monoterpenes on the feeding activity and food assimilation of *P. brassicae* larvae was studied in no-choice tests on leaf material of the host plant *Brassica oleracea*.

Material and Methods

Insects and plants. White cabbage *Brassica oleracea* L. var. *capitata* f. *alba* cv. Sława was grown under field conditions with no insect control measures applied. The L4 larvae of the large white butterfly *Pieris brassicae* (L.) and cabbage leaves were collected and transferred to the laboratory for the experiments.

Chemicals. The following 17 monoterpenoids were used in the study: 12 cyclic monoterpenoids (bornyl acetate, carvone, *p*-cymene, eucalyptol, (+)-fenchone, (+)-R-limonene, L-menthol, α-phellandrene, (+)-α-pinene, α-terpinene, γ-terpinene, thymol) and 5 acyclic monoterpenoids (citronellol, geraniol, (-)-linalool, α-ionone, β-ionone). All compounds were purchased from Sigma-Aldrich company.

Bioassays. Each monoterpenoid was tested in a 24-hour no-choice test with 10 replicates per test. The 40 mm x 40 mm fragments of cabbage leaves were immersed in 1% ethanolic solutions of individual monoterpenoids for approximately 3 seconds, dried, weighed and placed individually in Petri dishes of 10 cm diameter. Cabbage leaves immersed in 1% ethanol were used as control. The field collected L4 larvae of *P. brassicae* were weighed and placed in the Petri dishes containing the studied plant material (one larva/Petri dish). The experiment was carried out in the growing chamber Sanyo MLR-350H at 20°C and L14h:10hD photoperiod for 24 hours. At the end of the experiment, the plant material and the larvae were weighed.

Data analysis. The following parameters were calculated: amount of consumed food by the *P. brassicae* larvae, change in body weight of the larvae, and the absolute index of deterrence (DI) according to a formula: DI = (C–T)/(C+T) · 100, where C and T represent the amount of control and treated material consumed by the larvae, respectively. The data were subjected to statistical analysis using one-way ANOVA. The significance of differences in relation to control was estimated using the Dunnett's test at $p<0.05$. The data on the change in larval body weight were Box-Cox transformed ($\lambda = 4.99$, shift = 1.94). Additionally, the Tukey test (HSD) was used on the index of deterrence (DI) data to find significant differences in the activity among the studied compounds.

Results and Discussion

The significant reduction in the amount of the consumed food by *P. brassicae* larvae occurred after the application of α-phellandrene, β-ionone, α-ionone, α-terpinene, citronellol, (-)-linalool, and *p*-cymene (Table 1). The strongest deterrent effect was found in the case of α-phellandrene- and β-ionone-treated leaves: the larvae consumed approximately four times less food than in the control experiment. The least active compound *p*-cymene caused 1.5-fold reduction in the food consumption. The deterrence indices (DIs) for these substances ranged from 59.3 (α-phellandrene) to 21.2 (*p*-cymene). (+)-R-limonene, (+)-fenchone, γ-terpinene, and (S)-(+)-carvone had moderate but not significant antifeedant effect: the reduction of the feeding of *P. brassicae* larvae was approximately 1.4-fold. The DIs range was 21.1 ((+)-R-limonene) – 17.2 ((S)-(+)-carvone). The remaining compounds either did not show any effect on the food consumption of the larvae ((+)-α-pinene, eucalyptol, bornyl acetate; DIs: 9.2–1.3) or were very slightly attractant (geraniol, thymol, and L-menthol; DIs: -2.0 to -5.2) – Figure 1.

There were no significant differences in the change of body weight after the 24-hour experiment in caterpillars that were exposed to leaves treated with the studied monoterpenoids in comparison to control (Table 1). Nevertheless, the caterpillars that were offered leaves treated with α-phelandrene, β-ionone, and (-)-linalool showed almost no increase in body weight (14% of the control

Table 1
The effect of monoterpenoids on food consumption and assimilation of larvae of the *Pieris brassicae*

	Consumed food (g) mean (±SD)	Dunnett's test p	Change in body weight (g) mean (±SD)		Dunnett's test p
			non--transformed	Box-Cox--transformed	
Control	0.52 (±0.11)		0.07	6.41 (±0.79)	
α-phellandrene	0.13 (±0.04)	0.0000	0.01	5.46 (±0.20)	0.1377
β-ionone	0.16 (±0.05)	0.0000	0.01	5.45 (±0.27)	0.1232
α-ionone	0.27 (±0.11)	0.0008	0.05	6.06 (±0.54)	0.9913
α-terpinene	0.28 (±0.18)	0.0015	0.03	5.74 (±0.38)	0.5261
Citronellol	0.31 (±0.13)	0.0085	0.06	6.26 (±0.90)	1.0000
(-)-linalool	0.32 (±0.10)	0.0165	0.01	5.46 (±0.54)	0.1357
P-cymene	0.34 (±0.09)	0.0420	-0.04	5.69 (±2.08)	0.4425
(±)-R-limonene	0.35 (±0.14)	0.0717	0.05	6.14 (±0.52)	0.9996
(±)-fenchone	0.35 (±0.19)	0.0792	0.06	6.24 (±0.82)	1.0000
γ-terpinene	0.36 (±0.05)	0.0877	0.04	5.80 (±0.27)	0.8132
(±)-α-pinene	0.40 (±0.21)	0.3735	0.02	5.68 (±0.71)	0.4265
(S)-(±)-carvone	0.41 (±0.23)	0.4602	0.06	6.30 (±1.07)	1.0000
Eucalyptol	0.46 (±0.13)	0.9855	0.08	6.57 (±0.99)	1.0000
Bornyl acetate	0.50 (±0.07)	1.0000	0.05	6.05 (±0.71)	0.9886
L-menthol	0.50 (±0.09)	1.0000	0.09	6.73 (±0.56)	0.9971
Geraniol	0.57 (±0.22)	0.9983	0.09	6.84 (±1.27)	0.9572
Thymol	0.58 (±0.17)	0.9787	0.05	6.15 (±0.81)	0.9998

value). Additionally, the larvae that consumed leaves treated with *p*-cymene showed a decrease in body weight (1.6-fold reduction in relation to control). The food selection process by the caterpillars of the large white butterfly involves the response to plant surface chemicals that are detected by contact chemoreceptors (= taste receptors) on the mouthparts (SCHOONHOVEN, LIN-ER 1994). Glucosinolates, the characteristic chemicals of the Brassicaceae play a crucial phagostimulant role in this process (RENWICK, LOPEZ 1999, SMALL-EGANGE et al. 2007). The deterrent chemicals are also sensed by *P. brassicae* caterpillars at the plant surface level and cause the inhibition of the feeding activity (MESSENDORP et al. 2000).

Basically, an antifeedant is a behaviour modifying substance that deters feeding through a direct action on taste organs (Isman 2002). However, FRAZIER and CHYB (1995) suggested that insect feeding can be inhibited at three levels: preingestional (immediate effect associated with host finding and host selection processes involving gustatory receptors), ingestional (related

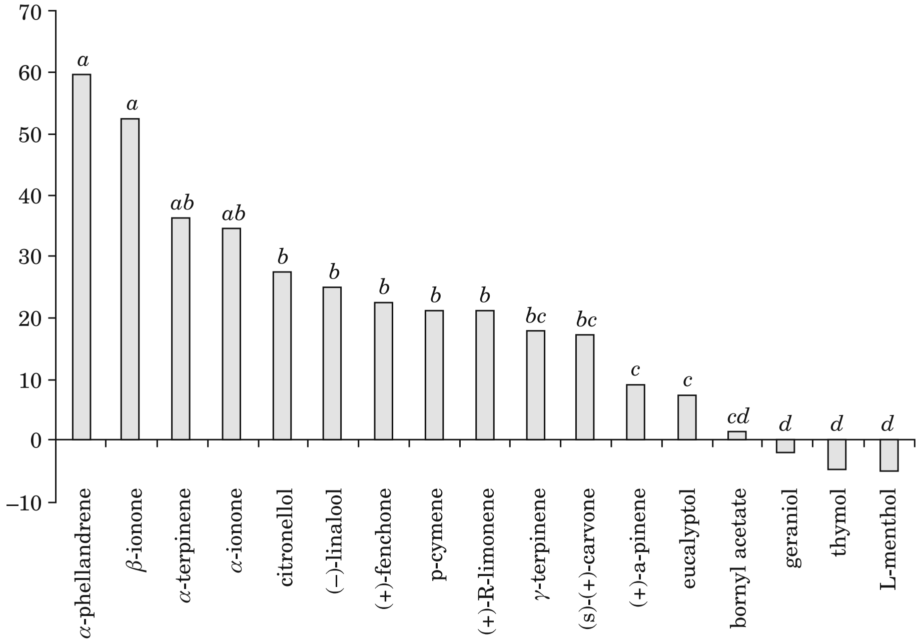

Fig. 1. Deterrence indices (DI) of monoterpenoids applied against larvae of the *Pieris brassicae*. Different letters show significant differences in activity among the terpenoids (Tukey's test at $p<0.05$)

to food transport and production, release, and digestion by salivary enzymes), and postingestional (long-term effects involving various aspects of digestion and absorption of food).

Conclusions

According to the comparative analysis of the feeding deterrence indices (DIs), all substances applied in the present study can be divided into five groups: highly active deterrents that practically completely inhibited the feeding of caterpillars (α-phellandrene and β-ionone; DI = 59 and 52, respectively), strong deterrents (α-terpinene and α-ionone; DI = 36 and 34), relatively strong deterrents (citronellol, (-)-linalool, *p*-cymene; DI = 27, 24, 21), moderate deterrents ((+)-fenchone, (+)-R-limonene, γ-terpinene, and (S)-(+)-carvone; DI = 22, 21, 17, 17), and inactive substances (h-pinene, eucalyptol, bornyl acetate, geraniol, thymol, and L-menthol). Moreover, α-phellandrene, β-ionone, α-ionone, α-terpinene, citronellol, (-)-linalool, and *p*-cymene may be classified as preingestive deterrents and *p*-cymene may be considered a postingestive deterrent.

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