Host-tree Preferences of the Pine Moth (Lepidoptera: Lasiocampidae) and Pine Beauty Moth (Lepidopera: Noctuidae) Larvae in Relation to Needle Quality

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Abstract

The larvae of *Dendrolimus pini* L. and *Panolis flammea* (Den. et Schiff.) usually occur in high numbers on different trees within a stand. Studies that focused on the host tree-preference of these two species were conducted in the Wymiarki Forest District (Poland) in 2001. Sixteen Scots pine trees were selected to estimate the larval abundance of both species using 1x1 m collectors. In June the trees were cut down, the larvae in crown were counted and the samples of needles for chemical analysis were taken from the lower, middle and upper parts of the crowns. Natural radiation was measured under each tree using the methods developed by dowsers (radiesthets). There were no significant differences in the concentrations of any terpene or phenolic acid in different parts of crowns. The abundance of pine moth larvae was related significantly with 7 monoterpenes, 6 sesquiterpenes and 5 phenolic acids, while the abundance of the pine beauty moth was related with 5 monoterpenes, 6 sesquiterpenes and 1 phenolic acid. There were 6 compounds which had significant impact on the abundance of both species: â-pinene was in positive relationship with *D. pini* and inverse with *P. flammea*, while the compound with a retention index of 1430, ã-muurolene, â-vetivenene, á-cadinene and protocatechuic acid were in inverse relationships with *D. pini* and positive with *P. flammea*. These differences may explain why these insect species are rarely observed in high numbers on the same trees. Natural radiation is possibly one of the causes of variation of secondary metabolites in Scots pine needles. It was in significant relationships with 9 monoterpenes, 3 sesquiterpenes and 1 phenolic acid. The results of studies suggest that â-pinene and sesquiterpene with a retention index of 1430 are the only links between natural radiation and abundance of both *D. pini* and *P. flammea*.

Key words: *Dendrolimus pini*, *Panolis flammea*, host preference, secondary metabolites, terpenes, phenolics, natural radiation

The pine moth, *Dendrolimus pini* L., and pine beauty moth, *Panolis flammea* (Den. et Schiff.), belong to a group of the most harmful defoliators of Scots pine, *Pinus sylvestris* L., in Poland. Both species have a one-year life cycle however their biologies are different.

The pine moth females lay eggs in July-August. Young larvae feed before late-October or early-November, overwinter in forest litter, and resume feeding in early spring on old needles. After all of the old needles are consumed, the larvae start to feed on young needles. The larvae pupate in June through August (Vorontsov 1982, Kolk and Starzyk 1996).

The pine beauty moth overwinters as a pupa. Adults emerge in April-May, mate, and females deposit egg masses. The young larvae emerge and begin feeding in late-May on buds, needles and bark of fresh shoots. The older larvae feed on old needles and pupate in late June-July.

Both species prefer 20-80 year old pine monocultures, however their outbreaks usually occur in different stands; if both species occur within a stand, high numbers of their larvae are observed on different trees. This observation led us to hypothesize that needle quality (concentration of secondary

metabolites) could be of a great importance in the host tree preference of the pine moth and pine beauty moth larvae.

On the other hand, the terpene components of coniferous resin systems are under strong genetic control and are valuable indicators of population diversity. Moreover, they interact with other components of the ecosystem and are thus of great value as adaptive markers (Forrest et al. 2000). All components of ecosystems are also under the impact of cosmic radiation, and geomagnetic, electric, electromagnetic and gravitational fields of the earth. Concentrations of minerals, underground streams of water, geological downcasts et al. cause the anomalies in these fields (Krolicki 1998), which may have an impact on the distribution of living organisms and their condition, biodiversity of ecosystems, and variability within a population.

Several studies on the impacts of different fields of the earth on some insect species were conducted in Austria and some other countries in the late 60s – early 80s of the last century (Schneider 1960, Robert 1963, Becker 1964, 1971, Schimitschek 1972, Jahn and Nessler 1971). Some studies by Jahn (1973, 1975, 1977) focused on the impact of natural radiation on nun moth survival and fecundity. Natural radiation was considered to be an electromagnetic radiation of very low frequency. The mortality of young nun moth larvae inside the zone of high radiation was significantly lower than in the normal zone. The mortality of young larvae reared on plants from the zone of high radiation was significantly lower and the fecundity of females was significantly higher than from those plants reared from the normal zone. These results suggest that there might be some direct relationships between the natural radiation of the earth and the occurrence of the defoliators or indirect through the impact of the radiation on the insect host trees. This hypothesis was also tested in our studies.

Material and Methods

Study Site

The studies were conducted in the Wymiarki Forest District of Regional Directorate of State Forests, located in the south western part of Poland. Scots pine stands (38-44 years of age) of similar quality and growing on similar site conditions, occur on an area of about 1500 ha. The threat of these stands from the pine moth and pine beauty moth in 2001 was assessed to be high, and therefore chitin synthesis inhibitors were applied on May 17 to prevent heavy defoliation of trees. The threat assessment revealed also that the pine moth was more abundant in some stands than in others while the pine beauty moth was more numerous in the stands where the abundance of pine moth was low. Insect abundance was estimated on 16 trees and samples of needles from each of the trees were taken for chemical analysis.

Estimation of Insect Abundance on Sampled Trees

One meter square collectors were placed beneath each of the selected trees at the time that the spray was applied so that dying insects fell into the collectors. One month later, on June 17, 2001, the trees were felled onto cotton sheets and the larvae of all species found in the crowns and on the sheet were counted.

Chemical Analysis of Needles

Chemical analysis was conducted to estimate the concentration of essential oils (monoterpenes and sesquiterpenes) and phenolics in the needles of the selected Scots pine trees. The samples of previous year needles were taken on June 17, 2001, from lower, middle and upper parts of crown of each tree in order to check the possible differences in the concentration of secondary metabolites among them. Analysis of the composition and concentration of monoterpenes and sesquiterpenes was conducted using GC/SPME and GC/MS methods. The concentration of phenolics was analyzed using the GC method. The analyses were conducted using HP 4890D chromatograph with FID detector (on DB-5 column, 30 m x 0,25 mm ID). The temperature program for analyzing phenolics was from 50 to

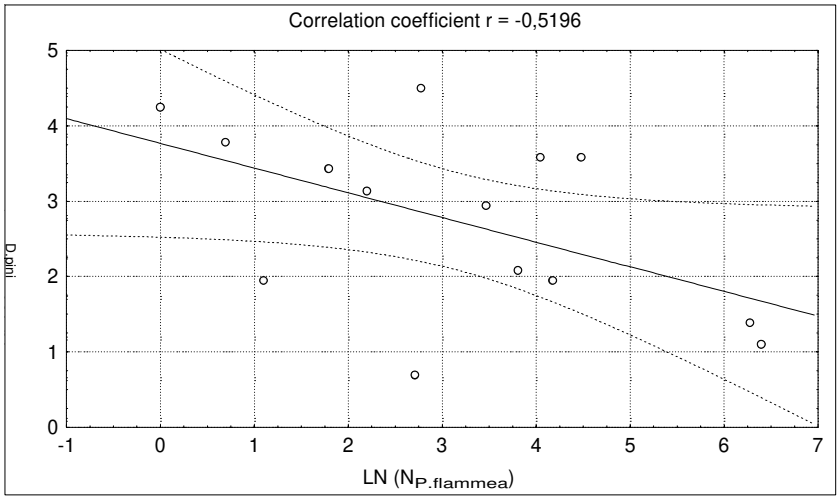

Figure 1.—The relationship between the ln-transformed abundances of the pine moth and pine beauty moth larvae on 16 selected Scots pine trees in the Wymiarki Forest District in 2001

300°C with a rate increase of 3°C/min. For essential oils, the program was from 40°C to 200° with a rate increase of 3°C/min followed by a rate increase of 10°C/min up to 300°C.

Measuring Natural Radiation

To determine the natural radiation, we used the method of Jahn (1974, 1977), which is known as the radiesthetical (dowsing) method. It is based on using a pendulum and scale specially developed by the radiesthets. In this case the natural radiation was measured in °SRW (Swaczyna 1991).

Statistical Analysis

Relationships between the concentration of each chemical compound and the number of specimens of each insect species, and the relationship between the natural radiation and the concentration of each chemical compound were analyzed with regression analysis using the statistical package Statistica 5.1/98 (StatSoft Poland). To decrease the variation of variables and to achieve a normal and homoscedastic distribution of points around the regression line, either or both variables were submitted to a logarithmic transformation.

Results

Insect Abundance

The number of the pine moth larvae on the trees varied from 0 to 90 specimens ($\overline{N}$ =24,6±25,93), while the number of the pine beauty moth varied from 0 to 598 specimens ($\overline{N}$ =94,4±185,18). There was an inverse relationship between the abundances of these two species on the selected trees (Fig. 1).

Secondary Metabolites

There were 28 monoterpenes, 29 sesquiterpenes and 18 phenolics in the needles sampled from 16 selected trees. The most common compounds which were used for the statistical analysis, are presented in Table 1. There were no significant difference among the concentrations of any compound measured in different parts of the crown, thus we used measurements from the middle of the crown in the data analysis.

Table 1.—Composition of the most common secondary metabolites in the needles sampled from 16 selected Scots pine trees in the Wymiarki Forest District, Poland, in June 2001.

Monoterpenes	Sesquiterpenes	Phenolics
3-hexene-1-ol	longicyclene	Vanillic
tricyclene	RI 1386	Homovanillic
RI 925*	β-longipinene	Protocatechuic
α-pinene	(E)-caryophyllene	Homogentisic
camphene	RI 1430	m-coumaric
sabinene	RI 1441	p-coumaric
β-pinene	(E)-β-farnesene	o-coumaric
myrcene	RI 1475	Gallic
3-carene	γ-muurolene	Phenol
α-terpinene	RI 1483	
limonene	β-selinene	
RI 1037	RI 1494	
(E)-β-ocimene	RI 1502	
γ-terpinene	(Z)-γ-bisabolene	
terpinolene	Â-vetivenene	
m-cymene	α-cadinene	
Bornyl acetate	RI 1581	
trans-sabinyl-acetate	Conipferyl alcohol	
RI 1340		
α-terpinyl-acetate		

*- undetermined compound with a retention index (RI)

Insect Abundance on Trees and Secondary Metabolites in Needles

Monoterpenes. Regression analysis of the concentrations of monoterpenes in needles and the abundance of the pine moth revealed that 7 monoterpenes were related to the number of the pine moth larvae per tree (Table 2).

One of them, β-pinene, was related positively with the abundance of pine moth larvae, while all of the others were related inversely. The strongest correlation between the variables was observed for camphene (r=-0.72), β-pinene (r=0.85) and trans-sabinyl-acetate (r=-0.80).

The abundance of the pine beauty moth larvae on trees was significantly and positively related to the concentrations of sabinene, RI 1037 and RI 1340 and inversely related with β-pinene and (E)-β-ocimene (Table 3).

Sesquiterpenes. Significant relationships were found between 6 sesquiterpenes and the number of pine moth larvae (Table 4), and 6 sesquiterpenes and the number of the pine beauty moth larvae on trees (Table 5). For the former species, all sesquiterpenes were inversely related with larval abundance and for the latter species, they were positively related with larval abundance.

Phenolics. Regression analysis revealed that the number of pine moth larvae on trees was significantly inversely-related with the concentrations of 4 phenolics extracted from the needles while positively related with gallic acid (Table 6). In case of the pine beauty moth, the only phenolic that was related to the abundance of larvae was protocatechuic acid and this relationship was positive [ln$N$ = 0.67 x ln (*protocatechuic acid*), r=0.71, R^2=0.46].

Table 2.—Regression models of the number of *Dendrolimus pini* larvae and monoterpenes in the needles of Scots pine trees (with p<0.05)

Monoterpene	Correlation coefficient	Regression model	Determination coefficient
camphene	-0.72	ln*N* = 4.7 - 0.87 x *camphene*	0.52
β-pinene	0.85	ln*N* = 1.51 x ln(*β-pinene*)	0.69
α-terpinene	-0.59	ln*N* = 4.2 - 11.79 x *α-terpinene*	0.35
γ-terpinene	-0.60	ln*N* = 4.6 - 6.49 x *γ-terpinene*	0.35
terpinolene	-0.59	ln*N* = 4.3 - 15.48 x *terpinolene*	0.35
m-cymene	-0.60	ln*N* = 4.3 - 0.64 x *m-cymene*	0.35
trans-sabinyl-acetate	-0.80	ln*N* = -1.21 x ln(*trans-sabinyl-acetate*)	0.59

Table 3.—Regression models of the number of *Panolis flammea* larvae and monoterpenes in the needles of Scots pine trees (with p<0.05)

Monoterpene	Correlation coefficient	Regression model	Determination coefficient
sabinene	0.71	ln*N* = 6.75 x *sabinene*	0.46
β-pinene	-0.69	ln*N* = 5.51 – 1.64 x ln (*β-pinene*)	0.48
(E)-β-ocimene	-0.68	ln*N* = 3.3 - 1.8 x ln (*(E)-β-ocimene*)	0.46
RI 1037	0.73	ln*N* = 46.56 x *RI 1037*	0.49
RI 1340	0.75	ln*N* = 0.35 + 12.26 x *RI 1340*	0.53

Table 4.—Regression models of the number of *Dendrolimus pini* larvae and sesquiterpenes in the needles of Scots pine trees (with p<0.05)

Sesquiterpene	Correlation coefficient	Regression model	Determination coefficient
RI 1430	-0.78	ln*N* = 4.65 - 12.26 x *RI 1430*	0.56
γ-muurolene	-0.66	ln*N* = 4.4 - 2.48 x *γ-muurolene*	0.39
RI 1494	-0.78	ln*N* = -1.76 x ln(*RI 1494)*	0.57
RI 1502	-0.85	ln*N* = 2.9 - 0.81 x ln(*RI 1502)*	0.70
β-vetivenene	-0.79	ln*N* = 5 - 0.57 x *β-vetivenene*	0.58
α-cadinene	-0.84	ln*N* = 5.2 - 18.5 x *α-cadinene*	0.67

Table 5.—Regression models of the number of *Panolis flammea* larvae and sesquiterpenes in the needles of Scots pine trees (with p<0.05)

Sesquiterpene	Correlation coefficient	Regression model	Determination coefficient
RI 1386	0.77	ln*N* = 6.85 + 1.58 x ln (*RI 1386)*	0.55
RI 1430	0.87	ln*N* = 27.71 x *RI 1430*	0.74
γ-muurolene	0.76	ln*N* = 7.27 x *γ-muurolene*	0.53
(Z)-γ-bisabolene	0.65	ln*N* = 1.45 x *(Z)-γ-bisabolene*	0.38
β-vetivenene	0.80	ln*N* = 1.55 x *β-vetivenene*	0.60
α-cadinene	0.85	ln*N* = 28.08 x *α-cadinene*	0.69

Table 6.—Regression models of the number of *Dendrolimus pini* larvae and phenolics in the needles of Scots pine trees (with p<0.05)

Sesquiterpene	Correlation coefficient	Regression model	Determination coefficient
Homovanillic	-0.74	ln*N* = 4.30 - 0.82 x ln (*homovanillic*)	0.55
Protocatechuic	-0.69	ln*N* = 4.96 - 0.42 x ln (*protocatechuic*)	0.47
o-coumaric	-0.76	ln*N* = 6.48 - 1.02 x ln (*o-coumaric*)	0.57
Phenol	-0.78	ln*Nl* = 7.46 - 1.49 x ln (*phenol*)	0.41
Gallic acid	0.70	ln*N* = 0.67 x ln (*gallic*)	0.61

Table 7.—Coefficients of correlation between the number of *Panolis flammea* and *Dendrolimus pini* larvae and chemical compounds related to both insect species

Type of chemicals	Compound	Correlation coefficient for	
		Dendrolimus pini	*Panolis flammea*
monoterpene	β-pinene	0.97	-0.69
sesquiterpene	RI 1430	-0.78	0.87
	γ-muurolene	-0.66	0.76
	β-vetivenene	-0.79	0.80
	α-cadinene	-0.84	0.85
phenolic acid	protocatechuic	-0.69	0.71

Among the compounds analyzed, 1 monoterpene, 4 sesquiterpenes and 1 phenolic acid were related to the abundance of larvae of both the pine moth and pine beauty moth (Table 7).

When the coefficients of correlation among these compounds and both insect species were compared, it was revealed that there was a difference in preferences of needles by larvae of the pine moth and pine beauty moth (Table 7). The abundance of pine moth larvae was positively related to â-pinene and inversely related to all other common compounds, while the pine beauty moth larvae preferred needles with a lower concentration of β-pinene and higher concentrations of other compounds.

Natural Radiation and Secondary Metabolites

Natural radiation measured under selected trees varied from 40 to 2100 °SRW ($\overline{R}$ =898,75832,16). There was a significant relationship between natural radiation and the concentration of some monoterpenes (Table 8), sesquiterpenes and 1 phenolic acid (Table 9) in the needles of selected Scots pine trees. These relationships were inverse for most monoterpenes, while positive for 3-hexene-1-ol, RI 1037 and (E)-β-ocimene, all 3 sesquiterpenes and homovanillic acid.

Discussion

The only studies on the relationship between monoterpenes and the pine moth larvae were conducted by Smeljanec (1969), and Rudnew and Smeljanez (1969). They revealed that camphene, â-pinene and borneol had positive effects on larval development while α-pinene, α-terpineol, limonene and bornyl acetate had negative effects. Our results are somehow contradictory as camphene was inversely related with the number of the larvae per tree, while the relationships with α-pinene, limonene and bornyl acetate were not significant. Only one compound, β-pinene, was positively related with the larvae of the pine moth in both studies.

Table 8.—Regression models of natural radiation and monoterpenes in the needles of Scots pine trees (with p<0.05)

Monoterpene	Correlation coefficient	Regression model	Determination coefficient
3-hexene-1-ol	0.77	3-hexene-1-ol = 0.042 x ln(*rad*)	0.55
RI 925	-0.65	*RI 925* = 0,34 - 0.036 x ln(*rad*)	0.37
β-pinene	-0.69	ln(*β-pinene)* = 3.48 - 0.38 x ln(*rad*)	0.47
3-carene	-0.70	*3-carene* = 57.8 - 6.42 x ln(*rad*)	0.49
α-terpinene	-0.66	*α-terpinene* = 0.24 - 0.022 x ln(*rad*)	0.43
RI 1037	0.82	*RI 1037* = -0.14 + 0.03 x ln(*rad*)	0.66
(E)-β-ocimene	0.74	ln(*(E)-β-ocimene)* = -2.63 + 0.43 x ln(*rad*)	0.54
terpinolene	-0.73	*terpinolene* = 0.26 - 0.03 x ln(*rad*)	0.53
m-cymene	-0.63	*m-cymene* = 4.04 - 0.34 x ln(*rad*)	0.40

Table 9.—Regression models of natural radiation and sesquiterpenes and phenolics in the needles of Scots pine trees (with p<0.05)

Compound	Correlation coefficient	Regression model	Determination coefficient
		Sesquiterpenes	
RI 1386	0.72	ln*(RI 1386)* = -4.13 + 0.4 x ln(*rad*)	0.47
RI 1430	0.82	*RI 1430* = -0.17 + 0.047 x ln(*rad*)	0.64
RI 1494	0.74	ln*(RI 1494)* = -4.35 + 0.29 x ln(*rad*)	0.50
		Phenolic acid	
homovanillic	0.70	ln*(homovanillic)* = 0.614 x ln(*rad*)	0.44

Studies on the effect of monoterpenes in Scots pine needles on the pine beauty moth were conducted by Leather (1987), however it concerned the oviposition preference by the female moths. The results of these studies suggest that eggs are laid more often on the needles of Scots pines which contained a high ratio β:α-pinene. This leads to an assumption that pine beauty females prefer to lay eggs on those trees which are most favorable for larvae of the next generation. Our results suggest that the needles of preferable trees should contain low amount of β-pinene and thus the ratio β:α-pinene should be low since there was no significant relationship between α-pinene and the number of the pine beauty moth larvae per tree.

The differences between the host tree preferences of *D. pini* and *P. flammea* larvae haven't been studied before. Six chemical compounds were identified that were significantly but oppositely related with the larval abundance of both insect species: β-pinene was related positively with *D. pini* but inversely with *P. flammea*, while the compound with a retention index of 1430, γ-muurolene, β-vetivenene, α-cadinene and protocatechuic acid were related inversely with *D. pini* and positively with *P. flammea*. These differences may help to explain why these two species are rarely observed in high numbers on the same trees.

The possible impact of natural radiation, though measured using a subjective method, on the chemical composition of the Scots pine needles and indirectly on insect occurrence was also investigated for the first time. A significant relationship was found for natural radiation and 9 monoterpenes, 3 sesquiterpenes and 1 phenolic acid. When natural radiation is included in a plant-

insect system, the interactions are getting more simple. The sesquiterpene with a retention time of 1430 and β-pinene seem to be the only links between natural radiation and abundance of both *D. pini* and *P. flammea*. Both compounds were inversely dependent on natural radiation, while they had different effects on the larval abundance of these species. These results suggest that natural radiation can have some impact on host plants and the larvae that feed upon them.

Further studies should focus on sampling more trees for analyses to confirm the results obtained so far and on using subjective and available objective methods to explore the relationships with natural radiation.

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