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Needle terpenoid composition may affect the infestation of the European larch by the larch wooly adelgid

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Abstract

The larch wooly adelgid *Adelges laricis* Vallot (Hemiptera Adelgidae) infestation has a damaging effect on the European larch *Larix decidua* Miller. In the present study, we found significant differences in *A. laricis* population number between individual larch trees. On the scarcely infested tree T1, few *A. laricis exulis progrediens* sessile nymphs/adult females and winged *sexuparae* occurred incidentally in mid-May and at the end of June. On the heavily infested tree T2, we found all developmental stages of *A. laricis* typical of the secondary host: the *exulis sistens* sessile nymphs/females, *exulis progrediens* sessile nymphs/females, L1 crawlers, and winged *sexuparae*. The population of *A. laricis* on T2 developed from the beginning of April, the maximum number occurred at the end of May, and the decline - in the end of July, which coincided with the migration of winged *sexuparae* to the primary host, the spruce *Picea abies* (L.) H. Karsten. We studied the qualitative and quantitative content of mono- and sesquiterpenoids in the needles of T1 and T2, as these allelochemicals play a major role in conifer defence against herbivores. Thirty mono- and sesquiterpenoids were identified using GC-MS. The amounts of verbenone, isobornyl acetate, and camphene were 42, 10, and 5 times higher in T1 than in T2, respectively. While the relative contents of verbenone and camphene were 3.4% vs. 0.0% and 6.0% vs. 1.8% in T1 vs. T2, respectively, the content of isobornyl acetate was 28% vs. 4.0%. It is likely that the relatively high content of isobornyl acetate in the needles is an important component of antixenosis resistance in *L. decidua* against *A. laricis*. The results of this study may contribute to future investigations on herbivore resistance in conifers and may appear helpful in designing sustainable control strategies in forests and in urban environments.

Key words: *Adelges laricis*, *Larix decidua*, terpenoids, isobornyl acetate, camphene.

Introduction

The larch *Larix* (Pinaceae) is one of the most common genera of coniferous trees in the Northern Hemisphere (Semerikov and Lascoux, 1999; Farjon, 2010). The trees of the genus *Larix* are unique among conifers because their needles are soft and deciduous, which means that they are shed every autumn (Vilcan *et al*., 2011). The European larch *Larix decidua* Miller is one of the most important species of the genus *Larix*. It is a component of natural forests in Europe where it grows in mixed and pure stands, mainly in the mountains up to 2500 m a.s.l. (Wagner, 2013; Da Ronch *et al*., 2016). It has been introduced to North America and New Zealand where it has a status of a naturalized species (Da Ronch *et al*., 2016). The European larch is also cultivated as an ornamental plant outside its natural localities, in parks and urban areas (Kulej, 2004; Szymański and Wilczyński, 2021). The wood of the European larch has long been used by humans, which made it a valuable tree species also in economic terms (Wagner, 2013; Da Ronch *et al*., 2016). Therefore, the spectrum of its insect pests, which belong mainly to the orders Coleoptera, Lepidoptera, Diptera, and Hemiptera, is relatively well known (Król and Skrzypczyńska, 1987; Schnaider, 1991; Skrzypczyńska, 1994; 2004; Rohde *et al*., 1996; Habermann, 2000). Within Hemiptera, the adelgids are considered serious pests of conifers in general and the European larch in particular (Varty, 1956; Hain *et al*., 1991; Young *et al*., 1995; Skrzypczyńska, 2004, Havill and Foottit, 2007; Cowles; 2009; Peccoud *et al*., 2010; Redfern, 2011; Havill *et al*., 2014; Limbu *et al*., 2018). Adelgidae, which are the sister group of aphids (Hemiptera Aphididae), include approximately 65 species originally distributed over temperate and boreal regions of the Northern Hemisphere where they infest exclusively gymnosperms and only the Pinaceae (Blackman and Eastop, 2000; Favret *et al*., 2015). Adelgids are oviparous (lay eggs) in all generations and typically exhibit multigenerational complex life cycles involving host-alternation between the primary host (*Picea* spp.) and the secondary host (*Abies* spp., *Larix* spp., *Pinus* spp., *Tsuga* spp., *Pseudotsuga* spp.). The sexual generation and gall formation occur on the primary host, while non-gall forming parthenogenetic generations occur on the secondary host. The entire life cycle takes two years, and involves five distinct generations (*sexuales*, *fundatrices*, *gallicolae* on primary host and *exules*, *sexuparae* on secondary host) (Havill and Foottit, 2007; Hain *et al*., 1991; Ponsen, 2006; Havill *et al*., 2007; Sano *et al*., 2008; Foottit *et al*., 2009; Redfern, 2011; Sano and Ozaki, 2012). Recently, the importance of the larch wooly adelgid *Adelges laricis* Vallot has increased. In Europe, it is recognized as the most dangerous pest of spruces and larches in natural forest environments and in artificial plantings in cities (Skarmoutsos, 1982; Brudea and Rîşca, 2010; Budzińska and Goszczyński, 2010; Osiadacz and Hałaj, 2011). The infestation of *A. laricis* often has a character of an invasion or an outbreak, which are especially damaging for young trees in nurseries as *A. laricis* impairs the tree assimilating apparatus, the needles (Brudea and Rîşca, 2010; Redfern, 2011; Havill *et al*., 2014; Limbu *et al*., 2018). The precise published data on *A. laricis* occurrence and population structure and dynamics in Poland and other European countries are barely available although the species is widely distributed (Szklarzewicz *et al*., 2000; Budzińska and Goszczyński, 2010; Osiadacz and Hałaj, 2011). Neither is there the precise information as yet, concerning the occurrence of this species on individual larch trees. In consequence, no data on the variation in susceptibility of the European larch to the infestation by *A. laricis* are available.

The resistance mechanisms of plants against herbivores are classified as antixenosis, antibiosis, and tolerance (Smith, 2005). Antibiosis occurs when the traits of a resistant plant have negative effects on the biology but not on the behaviour of an herbivore while antixenosis is based on the modification of herbivore behaviour by plant factors, which results in the inability of a plant to serve as a host. Tolerance is the ability of a plant to survive the infestation by herbivores and compensate the losses (Smith, 2005; Le Roux *et al*., 2008). Antixenosis and antibiosis in conifers depend on the morphological and anatomical structure of the plant and the presence of allelochemicals (Harborne, 1993). Terpenoids are the largest class of specialized plant natural products that form an essential part of constitutive and induced defence systems of conifers against herbivores and pathogens (Lewisohn *et al*., 1991; Phillips and Croteau, 1999; Croteau *et al*., 2000; Martin *et al*., 2004). From the plethora of terpenoid compounds synthesized by conifers, the blends of mono- and sesquiterpenoids constitute the first line of defence against insect folivores (Michelozzi, 1999; Ji and Ji, 2021). Generally, terpenoids are present in different tissues of a conifer plant: xylem, cortex and foliar tissue (Michelozzi, 1999). The terpenes in the needles are particularly important as the biosynthesis of individual compounds is under strong genetic control and is not significantly influenced by environmental factors (Mitic *et al*., 2017). The composition of terpenes in the needles of coniferous plants has been studied from various points of views: the variation within species and among individual plants (Keeling and Bohlmann, 2006; Semiz *et al*., 2007; Ji and Ji, 2021), the geographic and ecotype variation (Supuka and Berta, 1998; Pureswaran *et al*., 2004), the effects of the changing environment (Kylin *et al*., 2002; Kopaczyk *et al*., 2020), and in relation to biotic and abiotic stresses (Zou and Cates, 1997; Michelozzi *et al*., 1999; Montgomery and Lagalante, 2008; Kopaczyk *et al*., 2020). At the same time, the information on terpenes of the larch is very scarce and applies only to the terpenoid emission from different species (Von Rudlof, 1987; Abaimov *et al*., 1998; Kajos *et al*., 2013). Nevertheless, the existing reports reveal a variation in the qualitative and quantitative content of terpenes among different species of *Larix* (Von Rudloff, 1987; Abaimov *et al*., 1998; Ruuskanen *et al*., 2007; Ghirardo *et al*., 2010).

In the present study, we monitored in detail the dynamics and the age structure of *A. laricis* population on individual trees of *L. decidua* that we observed in our preliminary study as heavily or scarcely infested by the larch wooly adelgid. Considering the facts that mono- and sesquiterpenoids are the primary anti-herbivore defence allelochemicals in conifers and that the profile of terpenes typically varies within species or even among individual plants, we also investigated the qualitative and quantitative content of these chemicals in the needles of the studied trees.

Materials and methods

Research area and larch trees

The study was carried out in the allotment gardens ‘Przylesie’ in Sulechów (Lubuskie Province, Poland). The trees of the European larch *L. decidua* formed a row along the allotment border and they grew 1-2 m apart and in a close vicinity (3 m) of a row of spruces *Picea abies* (L.) H. Karsten. The larch trees neighboured also with the mixed forest across the field road along the border of the allotments (supplemental material figure S1). Two trees, T1 and T2 were selected for the monitoring of the larch wooly adelgid population and collecting the needles for terpenoid analysis. The trees T1 and T2 were identified as scarcely and heavily infested by *A. laricis*, respectively, during the preliminary study. For the sake of simplicity, we use the labels ‘adelgid-free’ for T1 and ‘adelgid-infested’ for T2 in the present study. The trees did not undergo any protective insecticide treatments.

Monitoring of *A. laricis* population development on *L. decidua*

The monitoring of *A. laricis* occurrence on *L. decidua* commenced on 2nd of April 2008, which coincided with the hatching of eggs that were laid by the *exulis sistens* females at the dwarf stem bases and was terminated on 24th of June 2008, when the winged *sexuparae* migrated to the winter host, the spruce. On T1 and T2, 25-cm long twigs were selected randomly (6-7 twigs approximately 1.6 m above the ground on each of the four sides of a tree; n = 25). The twigs were labelled and inspected in the morning hours from 8.00 to 11.00 every ten days. At each inspection time, the number of individuals and the developmental stages of *A. laricis* were recorded. The following *exulis* stages of *A. laricis* were identified: ESF - *exulis sistens* females (overwintering as nymphs at the dwarf stem bases), EPC - *exulis progrediens* crawlers (first instar nymphs of *progrediens* generation on needles), EPW - *exulis progrediens* advanced nymphs or adults covered with white, waxy wool (so-called ‘wooly’ instars on needles), and SW - winged *sexupara* females (winged adults without wool). Samples of nymphs and adults of *A. laricis* parthenogenetic generations from *L. decidua* were examined with the system microscope Olympus BX51/BX52. The photographs were taken with Olympus Camedia C-3030 ZOOM digital camera paired with Olympus DP-Soft 3.1 PC software.

Analysis of terpenoids

The needles for terpenoid analysis were collected by taking 25 uninfested twigs (15-20 cm long) at random from various branches of T1 and T2. The twigs were transported to the laboratory, where they were maintained at 4 °C until the sample preparation procedures. From each twig, a sample of needles was taken and individually cleaned with distilled water on a glass plate. For all analyses only the uninfested and undamaged needles were used. The needles were dried (65 °C, laboratory dryer - Wamed SUP-65) and kept in darkness at room temperature until the analysis.

The dried, powdered plant material (1 g) was placed into a round flask with 100 mL of distilled water.

Benzophenone (1 mg) was used as internal standard. The distillation-extraction process was performed in Deryng apparatus, using cyclohexane (1 mL) as the extracting organic solvent. After 1 hour of distillation-extraction process, the organic fraction was collected into Eppendorf tubes and frozen (−15 °C) until GC-MS (gas chromatography/mass spectrometry) analyses were performed. The isolation, identification and quantification of the volatile compounds were performed on a gas chromatograph, Saturn 2000 Varian Chrompack, with a column TRACE TR-1 30 m × 0.53 mm ID × 1.0 µm film. The mass spectrometer, equipped with an ion-trap analyser, was set at 1508 for all analyses with an electron multiplier voltage of 1350 V. Scanning was performed from m/z 39 to 400 in electronic impact (EI) at 70 eV, mode at 1 scan $second^{-1}$. Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL $minute^{-1}$ in a split ratio of 1:20 and a program: a) 80 °C for 0 minute; b) rate of 5.0 °C $minute^{-1}$ from 80 to 200 °C, rate of 25 °C $minute^{-1}$ from 200 to 280 °C and hold for 5 minutes. Injector and detector were held at 200 and 300 °C, respectively. 2 µL of the extracts were always injected. The system of identification of each compound included experimental and literature Kovats indices (KI). Most of the compounds were identified by using 3 different analytical methods: 1) Kovats indices MassFinder database, 2) GC-MS retention indices (authentic chemicals), and 3) mass spectra (authentic chemicals and NIST 20 spectral library collection). Identification was considered tentative when it was based on only mass spectral data. For the quantification of the volatile compounds benzophenone was added as internal standard (1 mg). All the terpene standards used for identification and quantification purposes were from Sigma-Aldrich.

Statistical analysis

The statistical analysis of *A. laricis* population development data included the descriptive statistics (means ± SE) of all variables. In addition, the numbers of *A. laricis* individuals on adelgid-free (T1) and adelgid-infested (T2) larches were compared using the Student t-test ($P = 0.05$). All statistical calculations were performed using StatSoft, Inc. STATISTICA (data analysis software system), version 13.3 www.statsoft.com.

Results

Population dynamics of *A. laricis* on *L. decidua*

The following developmental stages of *A. laricis* were recorded on the European larch: *exulis sistens* wingless females at dwarf stem bases (ESF) (figure 1a); *exulis progrediens* crawlers on needles (EPC) (figure 1b); *exulis progrediens* 'wooly' sessile nymphs and 'wooly' adult females on needles (EPW nymphs and EPW adults, respectively) (figure 1c) and winged *sexupara* females (SW) (winged adults without wool) (figure 1d).

Figure 1. Parthenogenetic developmental stages of *A. laricis* on *L. decidua*. (**a**) *Exulis sistens* female at dwarf stem base with a clutch of eggs (ESF). (**b**) *Exulis progrediens* mobile nymphs (= crawlers) (EPC) - 1st instar nymph after hatching on the larch needle, dorsal view on elliptical body. (**c**) Wax-covered *exulis progrediens* female (EPW) on larch needle. (**d**) Winged *sexupara* female on larch needle (SW). Olympus BX51/BX52; Olympus Camedia C-3030 ZOOM digital camera paired with Olympus DP-Soft 3.1 PC software.

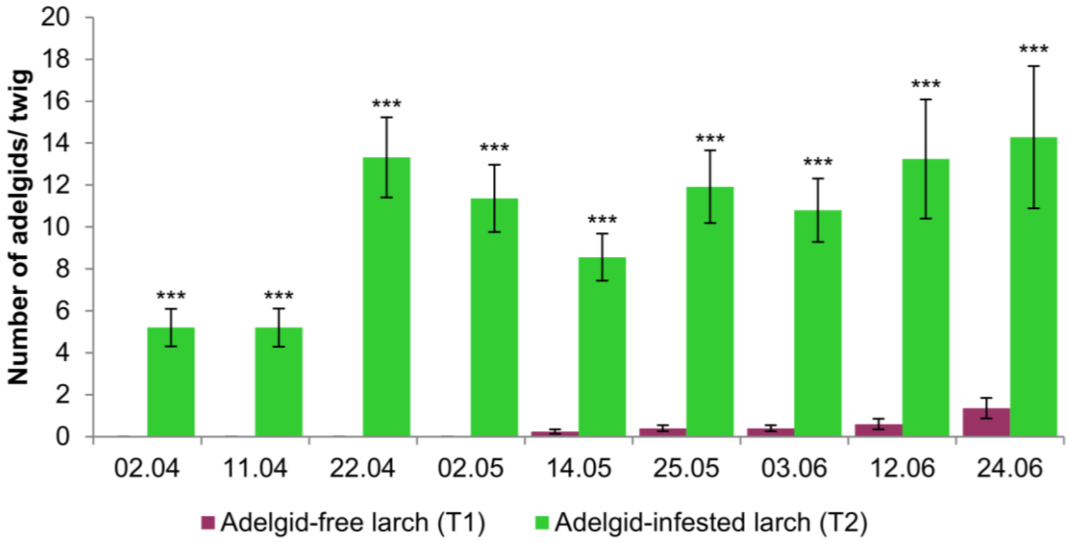

Figure 2. Occurrence of *A. laricis* on *L. decidua*. T1 - adelgid-free (= scarcely infested) tree, T2 - adelgid-infested (= highly infested) tree. Bars represent mean numbers of all recorded adelgid developmental stages per twig ± SE (n = 25). Asterisks represent statistically significant differences in the number of adelgids at each sampling date at p < 0.05 (Student t-test).

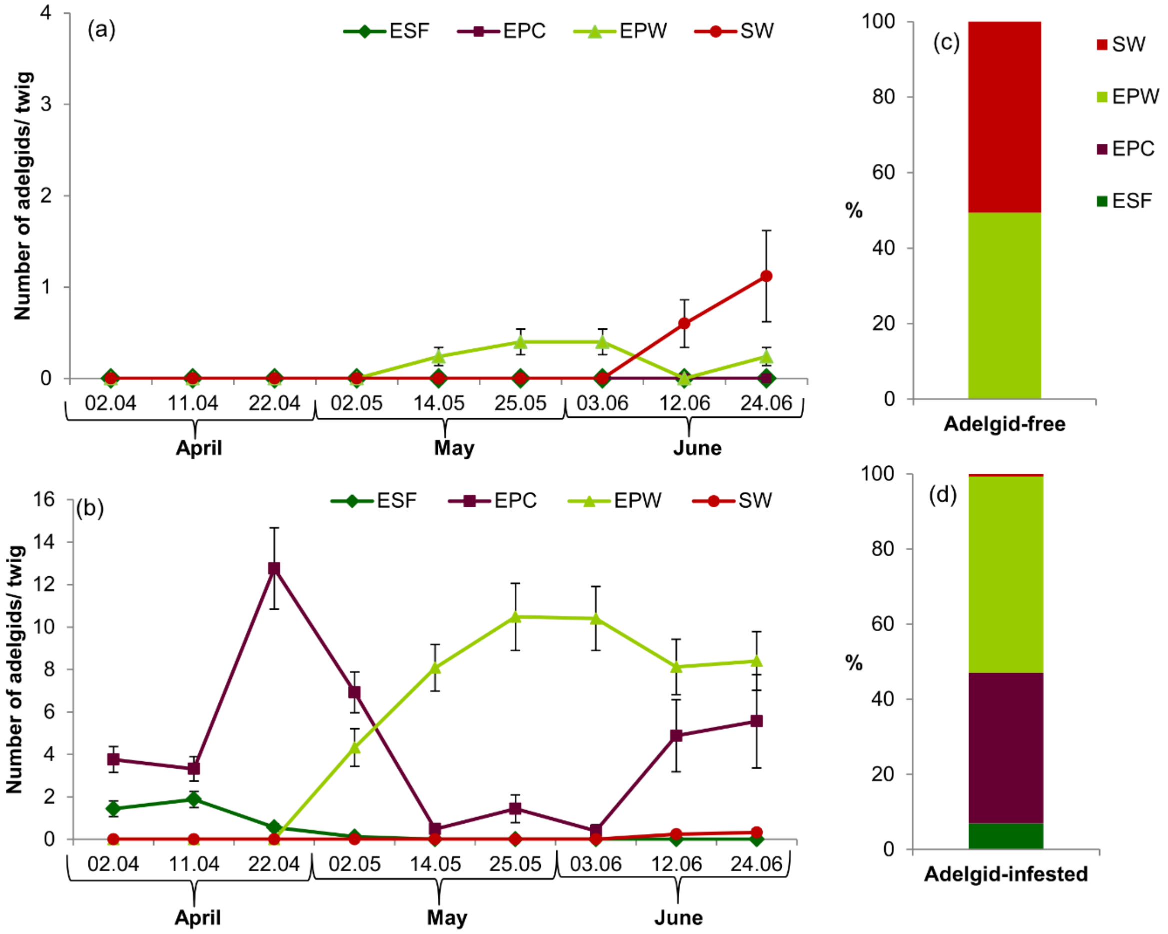

Figure 3. Dynamics of *A. laricis* population on *L. decidua*. (**a**) Population dynamics of *A. laricis* on T1. (**b**) Population dynamics of *A. laricis* on T2. (**c**) Population age structure of *A. laricis* on T1 (cumulative data for the whole study period). (**d**) Population age structure of *A. laricis* on T2 (cumulative data for the whole study period). ESF - *exulis sistens* females at dwarf stem base; EPC - *exulis progrediens* mobile nymphs (crawlers) on needles; EPW - *exulis progrediens* 'wooly' sessile nymphs or adults on needles and SW - winged *sexuparae*; T1 - adelgid-free (= scarcely infested) tree, T2 - adelgid-infested (= highly infested) tree.

The first adelgids were recorded on the 2nd of April on T2 while on T1 - on the 14th of May (figure 2, supplemental material table S1). On T1, the number of adelgids remained very low throughout the whole season and reached a maximum of 1.4 ± 0.5 individuals per twig on 24th of June. On T2, two peaks of *A. laricis* population occurred: on 22nd of April (13.3 ± 1.9) and on 24th of June (14.3 ± 3.4). At each inspection time throughout the monitoring period, significant differences in the total number of *A. laricis* on T1 and T2 occurred.

On T1, no ESF or EPC were observed throughout the whole research period. From the 14th of May to 24th of June 2008, the number of EPW remained low and ranged from 0.24 ± 0.10 to 0.40 ± 0.14 per twig. The occurrence of the first SW was registered on 12th of June, but they occurred also in a small number (0.60 ± 0.26 - 1.12 ± 0.50 per twig) (figure 3a, supplemental material table S1). During the entire observation period, the 'wooly' sessile individuals of EPW and winged females SW predominated and accounted for about 50% of *A. laricis* population on larch T1 (figure 3b, supplemental material table S1).

On T2, the occurrence of ESF was observed from 2nd of April until 2nd of May and the maximum of their number occurred on 11th of April (1.9 ± 0.4 individuals per twig on average) and then successively decreased. The EPC occurred throughout the whole observation period. The EPC numbers increased in April to reach the maximum on 22nd of April (12.8 ± 1.9 individuals per twig). When the number of EPCs began to decline, the first appearances of EPWs were recorded. The maximum of their number occurred from 25th of May until 3rd of June (10.5 ± 1.6 and 10.4 ± 1.5 individuals per twig, respectively) and then the number of EPCs decreased. From 12th of June, the number of EPCs increased again, but it remained at a lower level than in April (5.6 ± 2.2 individuals per twig on 24th of June). The occurrence of SW was registered on 12th of June, (figure 3c, supplemental material table S1). During the entire observation period, the 'wooly' sessile nymphs and females EPW predominated and accounted for more than 50% of *A. laricis* population on larch T2. The mobile nymphs EPC were also abundant (40.1%). ESF accounted for 6.9% of the population. The winged *sexupara* females SW accounted for less than 1% of the population (figure 3d, supplemental material table S1).

Table 1. Mono- and sesquiterpenoids identified in the *L. decidua* needles of *A. laricis*-free (= scarcely infested) tree T1 and *A. laricis*-infested (= highly infested) tree T2. RT - retention time; S - authentic standards; KI - Kovats index; MS - mass spectrum; tr - trace (below 1 μg/g).

	Compound	RT (min.)	KI exp.	KI lit.	Method of identification	Amount (μg/g)		Relative content (%)	
						T1	T2	T1	T2
1	*E*-3-hexen-1-ol	5.36	763	769	KI, MS, S	5.9	1.34	0.48	0.16
2	Tricyclene	7.22	924	927	KI, MS	5.4	5.71	0.44	0.68
3	α-Thujene	7.12	932	935	KI, MS	2.0	2.27	0.16	0.27
4	α-Pinene	7.44	934	936	KI, MS, S	198.3	211.34	16.12	25.16
5	Camphene	7.96	964	950	KI, MS, S	74.7	14.78	6.07	1.76
6	unknown	8.39			-	7.5	1.93	0.61	0.23
7	β-Pinene	8.68	973	978	KI, MS, S	23.9	33.60	1.94	4
8	β-Myrcene	8.53	982	987	KI, MS, S	9.3	8.48	0.76	1.01
9	3-Carene	9.39	1007	1010	KI, MS, S	30.6	24.02	2.49	2.86
10	α-Terpinene	9.56	1010	1013	KI, MS, S	8.9	6.97	0.72	0.83
11	p-Cymene	9.76	1015	1015	KI, MS, S	30.6	22.76	2.49	2.71
12	β-Phelandrene	10.04	1022	1023	KI, MS, S	17.8	33.68	1.45	4.01
13	γ-Terpinene	10.66	1048	1051	KI, MS, S	12.5	8.48	1.02	1.01
14	Terpinolene	11.5	1075	1082	KI, MS, S	16.1	9.74	1.31	1.16
15	α-Campholenal	12.75	1103	1105	KI, MS	12.4	12.94	1.01	1.54
16	*E*-Pinocarveol	13.38	1120	1126	KI, MS	25.5	55.86	2.07	6.65
17	Camphor	13.52	1129	1132	KI, MS, S	27.1	29.82	2.2	3.55
18	Isoborneol	14.02	1140	1142	KI, MS, S	57.4	54.10	4.67	6.44
19	δ-Terpineol	14.37	1152	1155	KI, MS, S	60.4	38.98	4.91	4.64
20	α-Terpineol	14.73	1175	1176	KI, MS, S	16.5	11.51	1.34	1.37
21	Myrtenol	14.93	1173	1178	KI, MS, S	15.5	18.65	1.26	2.22
22	Verbenone	15.27	1180	1183	KI, MS, S	41.8	tr	3.4	tr
23	Cuminal	16.18	1215	1218	KI, MS, S	3.9	5.88	0.32	0.7
24	Isobornyl acetate	17.24	1274	1276	KI, MS, S	342.4	33.43	27.84	3.98
25	*p*-Menta-1,4-dien-7-ol	18.52	1310	1315	KI, MS	8.6	11.68	0.7	1.39
26	*E*-β-Caryophyllene	21.25	1415	1421	KI, MS, S	11.2	10.01	0.91	1.19
27	γ-Cadinene	22.78	1505	1507	KI, MS	106.3	101.98	8.64	12.14
28	β-Cadinene	23.5	1521	1526	KI, MS	12.4	11.93	1.01	1.42
29	Caryophyllene oxide	25.49	1540	1546	KI, MS, S	8.5	12.26	0.69	1.46
30	ψ-Cadinol	26.24	1630	1633	KI, MS	16.7	23.02	1.36	2.74
31	α-Cadinol	26.54	1641	1643	KI, MS	19.8	23.27	1.61	2.77
	TOTAL					1229.9	840.42		

Mono- and sesquiterpenoids in *L. decidua* needles

In the uninfested needles from adelgid-free (T1) and adelgid-infested (T2) European larch trees, 31 mono- and sesquiterpenoids were detected, which included 17 monoterpenes, 7 monoterpenoids, 4 sesquiterpenes, 2 sesquiterpenoids and one undetermined compound (table 1). The monoterpenes were: acyclic (β-myrcene), monocyclic (campholenal, p-cymene, β-phelandrene, α-terpinene, γ-terpinene, terpinolene), bicyclic (camphene, camphor, 3-carene, isobornyl acetate, myrtenal, α-pinene, β-pinene, α-thujene, verbenone), and tricyclic (tricycene). The monoterpenoids included: acyclic (*E*-3-hexen-1-ol) and monocyclic (cuminaldehyde, p-menta-1,4-dien-7-ol, α-terpineol, δ-terpineol) and bicyclic (isoborneol, pinocarveol) compounds. Bicyclic sesquiterpenes embraced β-cadinene, δ-cadinene, t-caryophyllene, and caryophyllene oxide and bicyclic sesquiterpenoids - α-cadinol and psi-cadinol. Adelgid-free and adelgid-infested trees contained the same terpenoid compounds (table 1, figure 4).

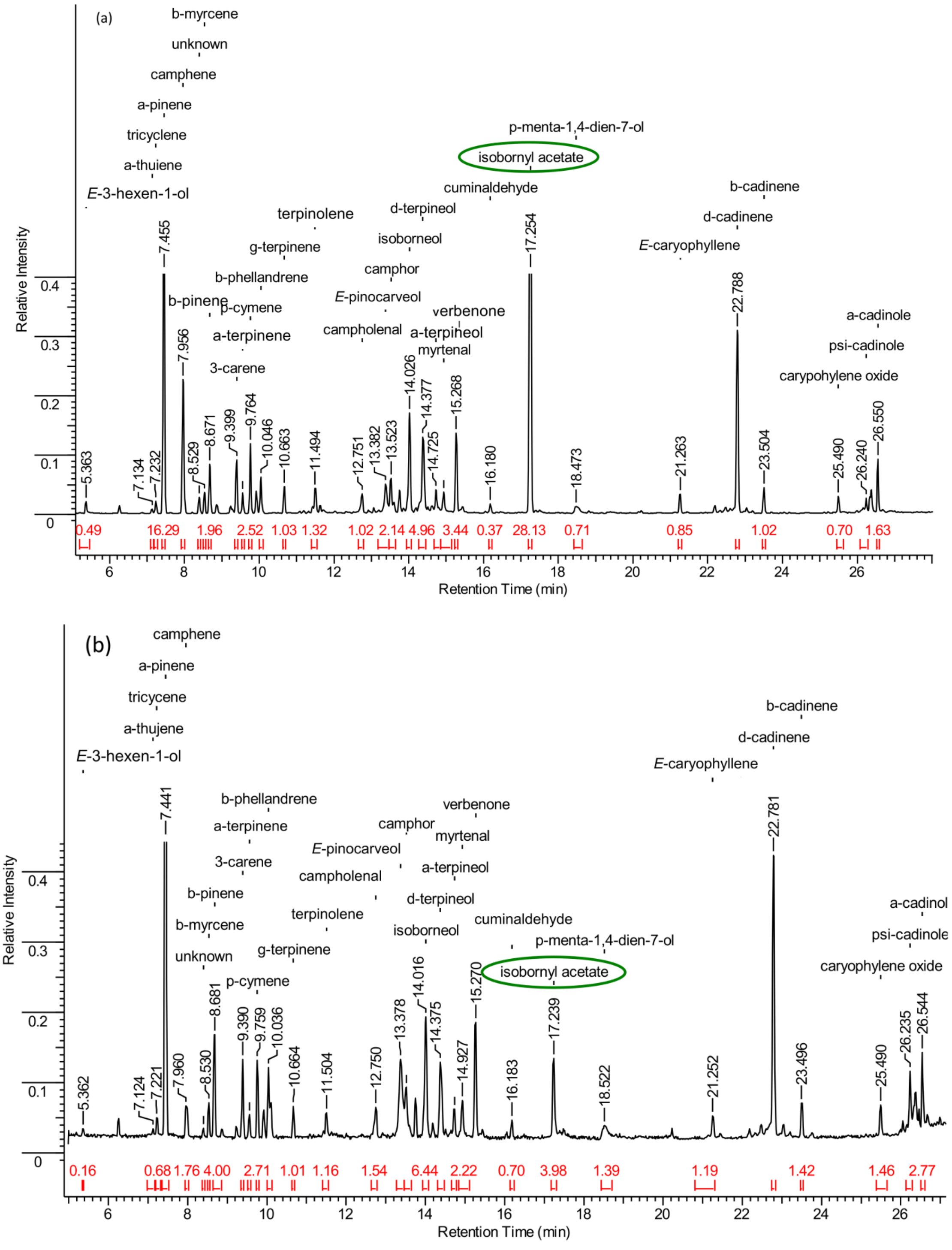

Figure 4. The GC-MS profiles of terpenoids of uninfested needles from *L. decidua*. (**a**) T1 - adelgid-free (= scarcely infested) tree; (**b**) T2 - adelgid-infested (= highly infested) tree.

The amounts of the majority of the recorded terpenoids were similar in uninfested needles from adelgid-free (T1) and adelgid-infested (T2) larches. The exceptions were verbenone, isobornyl acetate, and camphene, which were present in higher quantities (5.1, 10.2 and 41.8 times, respectively) in the adelgid-free T1 than in the adelgid-infested larch T2 (table 1, supplemental material figure S1). The relative content of these terpenoids indicated on the highest share of isobornyl acetate, camphene and verbenone (7.2, 3.5 and 3.4 times higher in T1 than in T2, respectively) (table 1, figure 4).

Discussion

The development of the larch wooly adelgid population on the European larch observed in the present study was typical for this species and all characteristic larch-associated developmental stages were recorded: *exulis sistens* females, *exulis progrediens* crawlers, 'wooly' sessile nymphs and adults, and winged *sexuparae*. The appearance and the sequence of individual generations reported on the European larch in our study agrees with the general description of the adelgid life cycle (Hain *et al.*, 1991; Ponsen, 2006; Havill and Foottit, 2007; Redfern, 2011). The first individuals of *A. laricis* that were recorded at the dwarf stem bases in the beginning of April were the *exulis sistens* females which had migrated to the larch the previous summer and overwintered. The rapid increase in the number of adelgids occurred at the end of April and in the beginning of May. It resulted from the high abundance of *exulis progrediens* crawlers that hatched from the eggs laid by the *sistens* females. A sharp decline in the number of crawlers occurred in mid-May, which coincided with an increase in the number of the succeeding developmental stages, the 'wooly' sessile nymphs and adults. Then, in mid-June, a gradual decline in the number of *A. laricis* on larch was observed. This decline was caused by the appearance of winged *sexuparae* which flied to the primary host (spruce), thus completing the cycle. Our study confirmed the previous reports that the turn of June and July is the typical migration period of *A. laricis* from the secondary host (larch) to the primary host (spruce) in Europe (Carter and Barson, 1973; Parry, 1973).

The typically developing population of *A. laricis* occurred only on one of the monitored larch trees, the T2 and not on T1, which validated our preliminary observations that motivated us to carry out the present study. *Exulis sistens* females and *exulis progrediens* crawlers did not occur on T1 throughout the whole season. Few *exulis progrediens* advanced nymphs or adults and winged *sexuparae* were recorded at the beginning of May. At that time, the population of *A. laricis* on T2 reached its maximum and first winged migrants started to appear. It is possible that some individuals of *A. laricis* from the adelgid-infested larch trees might have moved to the uninhabited tree, but they have not established a population there. Adelgid crawlers, which are the highly mobile 1st instar nymphs disperse within and between trees by crawling, being blown by the wind, or being transported by birds, deer, or other animals (Havill *et al.*, 2014). Several studies have shown that also aphid nymphs tend to move toward the twigs and that young nymphs have strong locomotor activity (Sandanayaka and Hale, 2003; Chen *et al.*, 2013; Hao *et al.*, 2020). Adelgid crawlers can disperse because they are highly mobile and they need fewer nutrients than the later stage nymphs and adults do (Dixon, 1998; Douglas, 2003; Havill *et al.*, 2014). Considering the fact that spruces from which *A. laricis* might have migrated in the previous autumn and the European larches heavily infested by *A. laricis* in spring grew close to each other, the almost complete absence of *A. laricis* on T1 tree was striking. The resistance of individual trees within a population to the infestation by adelgids has been reported for the European spruce *P. abies* to *Adelges abietis* (L.): although certain trees were regularly infested by the winged migrants, the population did not develop on these trees (Rohfritsch, 1988). Herbivore insects are discouraged from settling on plants mainly due to antibiosis (adverse effects on herbivore biology) and/or antixenosis (adverse effects on herbivore behaviour) (Fäldt *et al.*, 2002; Smith, 2005; Kopaczyk *et al.*, 2020). These mechanisms in conifers are mostly based on the variation in the terpenoid profiles, as specific monoterpenes have been shown to discourage insects, including the sap-sucking herbivores (Werner, 1995; Sadof and Grant, 1997; Powell and Raffa, 1999; Komenda and Koppmann, 2002). Adelgids have a limited tolerance of monoterpenes: high levels of santalene and camphor in the red spruce *Picea rubens* Sargent inhibit colonization by *Pineus floccus* (Patch) (Hemiptera Adelgidae) and high concentrations of limonene and myrcene in the Douglas fir *Pseudotsuga menziesii* (Mirbel) Franco are deterrent to *Adelges cooleyi* (Gillette) (Hemiptera Adelgidae) (Alexander, 1987; Stephan, 1987). Terpenoids 3-carene and β-pinene and sesquiterpenes may provide the Fraser fir *Abies fraseri* (Pursh) Poiret and the balsam fir *Abies balsamea* (L.) Miller with defence against the balsam woolly adelgid infestation (Carlow *et al.*, 2006). In aphids, the closest relatives of the adelgids, monoterpenes can reduce the reproduction in parthenogenetic individuals and inhibit oviposition in sexuals, and affect the wing dimorphism by stimulating the alate course of development (Harrewijn *et al.*, 1995; 2001). Four species of aphids associated with the Sitka spruce *Picea sitchensis* (Bongard) Carriere appeared to have a different level of tolerance to myrcene and piperitone that are present in the stem and needles (Jackson *et al.*, 1996).

In the present study, thirty terpenoids plus one unrecognized compound were identified in the uninfested needles from both adelgid-free and adelgid-infested European larch trees. The major difference between the studied trees related to the contents of camphene, verbenone, and isobornyl acetate. The absolute amounts of these compounds were respectively 5, 42, and 10 times higher in T1 than in T2. However, the content of isobornyl acetate was 28% vs. 4.0% in T1 and T2, respectively, while the relative contents of verbenone and camphene were 3.4% vs. 0.0% and 6.0% vs. 1.8%. Numerous *in vitro* and *in vivo* investigations have proven the biological activities of camphene that included antibacterial, antifungal, anticancer, antioxidant and insecticidal activities against polyphagous pests (Sharaby and El-Dosary, 2016; Thakare *et al.*, 2016; Benelli *et al.*, 2018; Hachlafi *et al.*, 2021).

Camphene reduced the total and mean probing time of the peach potato aphid *Myzus persicae* Sulzer (Hemiptera Aphididae) and limited the aphid settling on the leaves (Gabryś *et al*., 2005; Dancewicz *et al*., 2016). The essential oil from the roots of *Valeriana officinalis* L. (Valerianaceae) and its two major constituents bornyl acetate (48.2%) and camphene (13.8%) showed the promising fumigant and contact toxicity against *Liposcelis bostrychophila* Badonnel (Psocotptera Liposcelididae) and repellent effect on the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae) (Feng *et al*., 2019). The essential oil obtained from the leaves of *Chamaecyparis obtusa* (Siebold et Zuccarini) Endlicher (Cupressaceae), rich in isobornyl acetate showed relatively strong antibacterial activities against Gram (+) bacteria and some fungi (Yang *et al*., 2007). The essential oil of *Schizogyne sericea* (L.f.) de Candolle (Asteraceae), which contained p-cymene, isobornyl acetate and thymol esters, had insecticidal activity against larvae of *Culex quinquefasciatus* Say (Diptera Culicidae) and adults of *M. persicae* (Benelli *et al*., 2019). Isobornyl acetate and α-humulene in the needles of hemlock are presumably linked to the resistance of hemlock to the hemlock woolly adelgid (Montgomery and Lagalante, 2008).

In summary, we determined that the population of the larch wooly adelgid on larch in western Poland develops from the beginning of April until late June, with a maximum number in mid May. We confirmed that the dispersal of *A. laricis* on a larch tree starts with the intense within-tree migration of 1st instar nymphs, the crawlers, that hatch from eggs laid by females that arrive to the larch the previous autumn. In mid-spring, *alatae* migrants that develop on larch may also contribute to an increase in further infestation of trees. We determined that individual trees of the European larch differ in susceptibility to the infestation of the larch wooly adelgid. We revealed that the composition of mono- and sesquiterpenoids in the needles of adelgid-free and adelgid-infested larch trees was similar but the trees differed in the quantity of individual compounds. The population of *A. laricis* did not develop on the tree with sevenfold higher relative content of isobornyl acetate in the needles. The high abundance of isobornyl acetate in larch needles may be an important part of the constitutive defence mechanism in the European larch against the larch woolly adelgid. Nevertheless, more detailed studies will be needed to definitely establish the effect of lower terpenoids on *A. laricis*. Specifically, behavioural studies may provide evidence whether isobornyl acetate elicits the avoidance behaviour in adelgids when it is tested individually. Major allelochemicals are not always responsible biological activity when occurring individually. The blends of compounds often show a synergistic effect of the constituents (Miresmailli *et al*., 2006). The present results provide valuable practical information on antixenosis resistance in *L. decidua* that may be applied by the future researchers and growers in the control of *A. laricis*.

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