Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Composition of fatty acids in hemp leaves (*Cannabis sativa* L.) under the impact of aphids and a herbicide

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DOI: 10.24425/jppr.2025.155051

Received: March 02, 2024
Accepted: January 08, 2025
Online publication: July 01, 2025

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Responsible Editor:
Milan Brankov

Abstract

Cannabis aphid *Phorodon* (*Diphorodon*) *cannabis* Passerini 1860 is an economically important pest of oil hemp (*Cannabis sativa* L.) and is controlled by insecticides. Oil hemp crops are treated with herbicides, which are non-target pesticides for aphids but may also affect aphid populations. Such ecological implications of plant protection products are rarely investigated. The aim of the present research was to better understand plant – aphid – herbicide interactions, specifically, changes of fatty acids (FAs) in leaves, caused by cannabis aphids and a common herbicide used in hemp fields.

Of 21 FAs detected in hemp leaves, aphid feeding significantly increased the amounts of myristic and oleic acids and decreased the content of α-linolenic acid. This effect was found when aphids fed on hemp plants and especially when plants were treated with an herbicide containing quizalofop-P-tefuryl. This compound on its own did not affect the FA composition. In spite of the extremely high increase of myristic acid (7- to 9-fold, depending on the experiment variant), which could cause the repellent effect in hemp plants, the decreased amount of α-linolenic acid, the precursor of jasmonic acid may have helped aphids to manipulate the jasmonate signaling pathway involved in plant defense to herbivory enabling their continued feeding on hemp. This study revealed the importance of FAs in plant defense as well as the side effects of non-target plant protection products. Future pest management should take into account the complex interactions between crop plants, their pests and non-target effects of chemicals used in real field situations.

Keywords: ATR-FTIR spectroscopy, cannabis aphid, fatty acids, jasmonate pathway, non-target pesticide, oil hemp

Introduction

For centuries hemp/cannabis has been greatly valued for its fiber, seeds, and plant extracts, primary cannabinoids and terpenes (Bakro *et al.* 2020; Cantele *et al*. 2020; Farinon *et al*. 2020; Leonard *et al*. 2020). Hemp is also used for oil and great attention has been paid to the fatty acid composition in seeds. Lipids and fatty acids are essential constituents of all plant cells. They are major components of membranes and are therefore responsible for compartmentation of cells, by forming hydrophobic barriers (Kim 2020). Lipids are used as an energy source for seed germination and provide energy for various metabolic processes. Moreover, lipids and fatty acids also act as intracellular and extracellular signal transduction mediators (Lim *et al.* 2017). Oil extracted from hempseed contains more than 80% unsaturated fatty acids (Callaway 2004; Golimowski *et al.* 2022). In oil hemp, the omega-6 : omega-3 ratio ranges from 2 : 1 to 3 : 1. These values are

considered optimal for human health (Lupette and Benning 2020). This is why the first novel oil hempseed cultivars, including cultivar Henola used in this study, gained great attention and are regarded as attractive alternatives to other plants containing unsaturated fatty acids. In contrast to the seeds, not much is known about the contents of fatty acids in hemp leaves (Lemberkovics *et al*. 1979). The volatile oils and resin in leaves and flowers contribute to insect attraction or deterrence (Truta *et al*. 2009). In this study it was hypothesized that aphid feeding and pesticide use change the composition of fatty acids in hemp leaves.

The activity of fatty acids towards insects greatly depends on their structure (Juárez and Napolitano 2000). Fatty acids are divided into saturated (SAFA) and unsaturated (UFA), which can be further divided into monounsaturated fatty acids (MUFA), such as 18:1 (oleic acid) and polyunsaturated (PUFA), e.g., 18:2 (linoleic, LA), and 18:3 (α-linolenic, ALA). Unsaturated fatty acids (UFAs) are importantly associated with abiotic and biotic stresses (Harwood 1988) and play regulatory roles in plant defense (Lim *et al*. 2017). Inside cells they serve as intrinsic antioxidants and precursors of various bioactive molecules, such as the well-known stress hormone jasmonic acid, JA (He *et al*. 2018). The involvement of oleic acid in the crosstalk between JA and salicylic acid (SA) signaling pathways against pathogen invasion has been demonstrated (Kachroo *et al*. 2001). PUFAs are produced in plants through two parallel pathways, with one involving stearate desaturation to produce oleate (18:1), which is further desaturated to produce LA and ALA (Kanobe *et al*. 2015). Their oxidation leads to the synthesis of oxylipins (oxidized lipids), which function as signaling molecules in response to biotic stresses (Blée 2002; Prost *et al*. 2005). Their role in cross-kingdom communication between plants and pathogenic fungi was implicated (Christensen and Kolomiets 2011) as well as their involvement in defense against insect pests (Farmer *et al*. 2003).

A wide range of pests can cause damage to hemp such as loss of biomass, seed yield as well as the reduction in quality of harvested produce (McPartland *et al*. 2000; Bakro *et al*. 2018) The cannabis aphid *Phorodon* (*Diphorodon*) *cannabis* Passerini 1860, is a major pest of *Cannabis sativa* worldwide, including Poland (Durak *et al*. 2021). Damage is caused by sucking phloem sap from leaves and stems, leading to yellowing and dieback and can lead to significant yield loss in hemp (Cranshaw *et al*. 2018). It is distributed from central, eastern and southern Europe to Turkey, Asia, North Africa and North America (Heie 1994; Blackman and Eastop 2024). Cannabis aphid has a monoecious holocyclic cycle and its development is optimal at 25°C; the average fecundity at this temperature is 7.5 nymph per female per reproduction day (Durak *et al*. 2021).

As herbivores, aphids pose a special challenge to plants as they cause relatively little direct tissue damage, when inserting their slender stylets intercellularly to feed from the phloem sieve elements. This feeding strategy requires the recognition of aphid salivary components and the induction of phloem-specific defense (de Vos *et al*. 2007). The composition of phloem may change in response to aphid feeding and deter the pest or at least negatively affect its fecundity and other biological parameters. However, the insect-plant coevolution has resulted in the development of an ability in insects to counteract. The salivary effector proteins are known to suppress plant defense, as has been demonstrated in green peach aphid, *Myzus persicae* Sűlzer (Bos *et al.* 2010; Pitino and Hogenhout 2013; Elzinga *et al*. 2014) enabling the insect to feed on host plants.

The composition of phloem sap in crop plants is also affected by other factors such as pesticides which play an important role (Bromilow *et al*. 1990). Ecological implications of plant protection products are of great importance and interest due to the fact that they relate to phenomena found in agricultural fields. It was hypothesized that foraging of cannabis aphid on plants treated with a pesticide affects the composition of fatty acids in leaves. The studied chemical compound contained quizalofop-P-tefuryl, an active ingredient in an herbicide designed for controlling monocotyledonous weeds in dicotyledonous crops. This active substance is widely used to kill unwanted grasses in hemp fields by inhibition of Acetyl CoA carboxylase (https://hracglobal.com). This compound causes a mild stress in aphids foraging on treated plants and increases their reproduction (Durak *et al*. 2021). In this experiment it was assumed that hemp plants would respond to aphid feeding with fatty acid reprogramming. The kinetics of quizalofop–P–tefuryl herbicide was determined to check its presence in the phloem sap. To help interpret the results this included an additional explanatory study in which the concentration of quizalofop in plants during aphid feeding was determined.

Materials and Methods

Aphids and plants

Six-week-old glasshouse-grown hemp plants (*Cannabis sativa* L.) cv. Henola (Institute of Natural Fibres and Medicinal Plants, Poznań, Poland) which had 10–12 fully developed leaves were deliberately colonized by the laboratory population of cannabis aphids. There were four treatments in the experiment: 1) control plants, P, 2) plants treated with herbicide containing quizalofop–P–tefuryl, P+H, 3) plants infested with aphids, P+A, 4) plants treated with herbicide and infested with aphids, P+A+H. Control plants were not

treated with any pesticide or aphids. To determine aphid population growth over time, 24 hours prior to aphid release hemp plants were treated with the herbicide quizalofop-P-tefuryl (Pantera 40EC, Arysta Life Sciences, Amsterdam, The Netherlands). The concentration of the herbicide was prepared according to the recommendations of the manufacturer (40 g · l^{-1}, 4.38% in water). The solution was applied with a soft brush to make sure that all plant parts were uniformly covered with the same amount of the compound.

Experiment 1. Development of the population of cannabis aphid

Twenty-four plants were used in this experiment, 12 treated with the herbicide and 12 control (untreated) plants. The aphids were placed on plants 24 hours after herbicide application, when the herbicide had been absorbed by the plant and the plant was not wet.

Using a fine brush 35 adult apterous aphids were carefully placed on the upper leaves of each hemp plant. The population of aphids (population propagated in the laboratory) was counted 3, 5, 7 and 10 days after their release on plants. For each time variant, the number of nymphs born was counted on three control plants and three plants treated with the herbicide. These plants were then eliminated from the study (Fig. 1).

Experiment 2. Determination of fatty acids in plants with cannabis aphid foraging

There were four variants in this experiment, with 21 plants in each variant (84 plants in total). Variant 1: plants with no treatment (P), variant 2: plants treated with the herbicide (P+H), variant 3: plants treated with aphids (P+A), variant 4: plants treated both with herbicide and aphids (P+H+A) (Fig. 2).

The herbicide was applied on plants and 24 h later the aphids (35 apterous adults) were applied and this plant status was regarded as the starting point (0 h). Plant samples (leaves) for biochemical analysis were taken at 0 h (herbicide treatment only) and then 24 h, 48 h, 72 h, 96 h, 168 h (7 days) and 240 h (10 days) after herbicide treatment as well as the herbicide treatment and aphid application. Aphids were removed from each plant and counted. The leaves (without aphids) were frozen with liquid nitrogen and then stored in

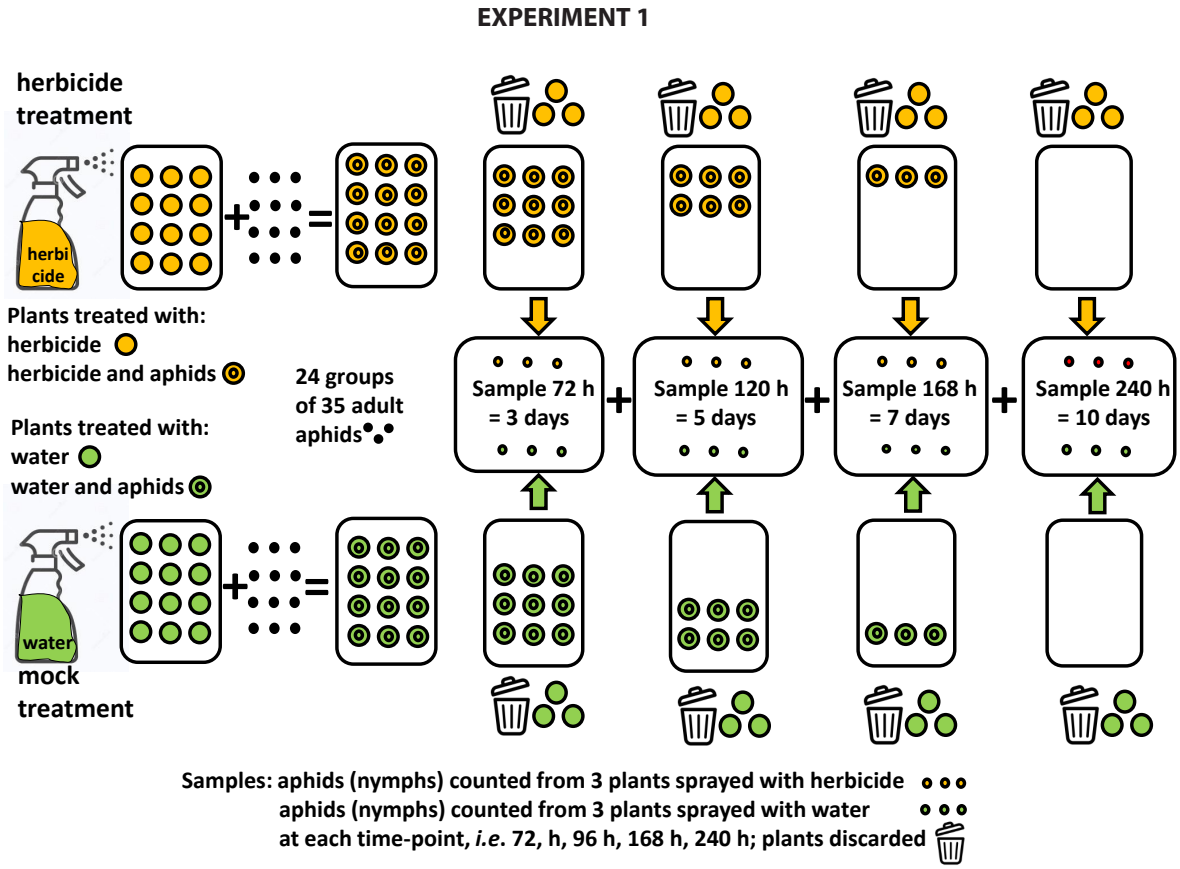

Fig. 1. The scheme of Experiment 1: hemp (*Cannabis sativa*) plants were treated with the herbicide containing quizalofop-P-tefuryl or with water and 24 h later with cannabis aphids (*Phorodon cannabis*). The population of aphids was counted 3, 5, 7 and 10 days after treatment; counted plants were removed from the experiment

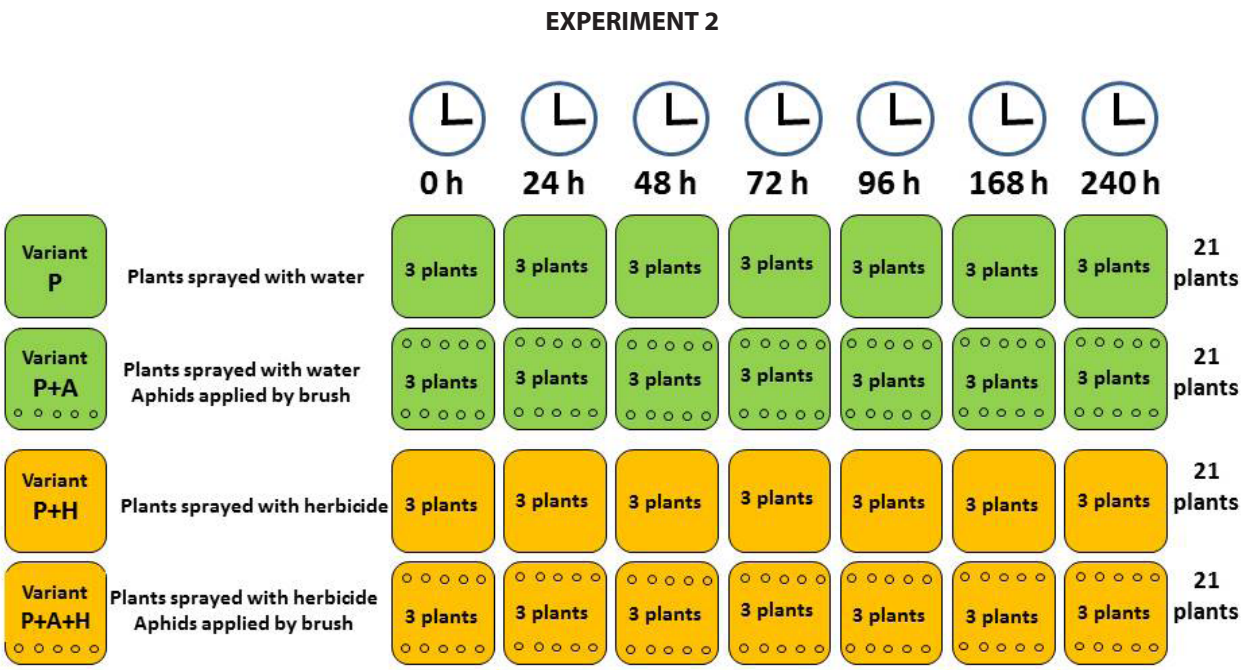

Fig. 2. The scheme of Experiment 2: hemp (*Cannabis sativa*) plants were treated with water (P) or herbicide (P+H) and infested with aphids *Phorodon cannabis* (variants P+A, P+A+H, respectively). The other half was left as controls. Plant samples were collected after 24 h (1 day), 48 h (2 days), 72 h (3 days), 96 h (4 days), 168 h (7 days) and 240 h (10 days). Aphids were then counted and removed. The content and composition of fatty acids (SAFA, MUFA and PUFA) were measured separately for each time point (in 3 replicates) and compared with the initial amount of fatty acids of each detectable type (0 h)

a deep freezer at −80°C. Each variant had three replicates (plants) per each of the time-points: 0 h, 24 h, 48 h, 72 h, 96 h, 168 h, 240 h (21 plants per variant).

Measurement of quizalofop-P-tefuryl herbicide residues

Quizalofop-P-tefuryl is the derivative of arylphenoxypropionic acids (FOPs), the compound which is the active ingredient in Pantera 40EC, a graminicide used to control weeds in hemp fields. According to the Herbicide Resistance Action Committee (HRAC), the product belongs to group A, which acts by the inhibition of Acetyl CoA carboxylase. The compound blocks the synthesis of lipids in monocotyledonous plants, with the first symptoms observed within a week and full effect 2–3 weeks after the application, depending on the age and size of weeds as well as the environmental conditions. The graminicide does not affect dicotyledonous plants.

Residues of quizalofop-P-tefuryl were determined in the hemp leaf samples used for the evaluation of FA composition. They were extracted at 0h, 24 h, 48 h, 72 h, 96 h, 168 h (7 days) and 240 h (10 days) after treatment by acetone: hexane mixture (1 : 9 v/v) (Storelli 2014). For each replicate 100 mg of dry leaf material was placed in a 1.5 ml Eppendorf tube, then 1 ml of acetone:hexane mixture (1 : 9 v/v) was added and shaken by vortex for 10 min (Bench MixerTM BV 1000, Benchmark Scientific Inc., Edison, NY, USA). Samples were centrifuged at 5000 rpm for 10 min (centrifuge 5804R, Eppendorf, Hamburg, Germany). The determination of quizalofop-P-tefuryl was carried out using a gas chromatograph (Agilent Technologies, model 7890A, Palo Alto, CA, USA) connected to a triple quadrupole mass detector (GC–MS/MS QqQ, Agilent Technologies, model 7000, Palo Alto, CA, USA). The certified analytical standard of quizalofop-P-tefuryl (Merck KGaA, Darmstadt, Germany) was used at 100 $\mu g \cdot ml^{-1}$ concentration. Data acquisition and processing was carried out by Mass Hunter ver. B.04.00 software. The extracts were separated on a HP-5MS Ultra Inert column (30 m × 0.25 mm ID × 0.25 µm). Prior to the chromatographic analysis, internal standard triphenylphosphate (TPP) was added to samples. Samples were analyzed in the Dynamic Multiple Reaction Monitoring mode (dMRM) (Słowik-Borowiec *et al.* 2022). The determination was carried out for fragmentation: 428.2 → 299.1 (10 eV), 428.2 → 285.1 (10 eV), 163 → 136 (10 eV), 163 → 100.1 (20 eV) (Document No SANTE 2021). Linearity for quizalofop-P-tefuryl expressed as determination coefficient was 0.996, and recovery at the level 0.001 ppm was equal to 101.3 ± 12.0% while it was 99.8 ± 0.6% at 0.1 ppm. The dissipation trends were calculated according to the following equation:

$$y = y0 \times e^{-kt},$$

where: y represents the residue concentration at the time t (mg · kg^{-1}), $y0$ represents the initial residue concentration at the time zero, $t = 0$ (mg · kg^{-1}), t – time, and k represents the degradation rate constant (Podbielska *et al*. 2023). From the above formula, the half-life for tested active ingredient was calculated using the formula: $t\,½ = \ln 2\, k^{-1}$.

Application of ATR-FTIR spectroscopy

Evaluation of the effect of aphid feeding on fatty acid content in hemp control plants (untreated) was done using the Attenuated Total Reflectance (ATR) sampling method for Fourier Transform Infrared (FTIR) spectroscopy (Smith 2011). To compare the ATR-FT-IR spectra, hemp leaf samples were collected from the same experimental batch, three individual plants at three sampling points at: 0 h, 168 h (7 days) and 240 h (10 days). The plant material was dried at 60°C for 72 h according to the Cornelissen protocol (Cornelissen *et al*. 2003) and ground to a fine powder using a laboratory ball mill MM400 (Retsch Polska, Katowice, Poland). Drying at this temperature is effective in preserving the fatty acids and does not alter the relative fatty acid composition in biological material (McCauley *et al*. 2016; Dobermann *et al*. 2019). Then 1.2 mg of the material was placed on the ATR-FTIR crystal and clamped using pressure gauges. Spectra of samples were measured using a Nicolet iN10 MX microspectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and measurements were analyzed using OMNIC software (Thermo Fisher Scientific Polska, Warsaw, Poland). Spectra for background and sample measurements were recorded at a resolution of 2 cm^{-1} and 64 scans in the range of 525–4000 cm^{-1}. Each spectrum was calculated as an average of three corresponding sample measurements to reduce the impact of intraspecific chemical composition variability on the results. Spectra were analyzed with baseline correction and vector normalization using OPUS 7.0 software (Bruker Optik GmbH, Ettlingen, Germany).

Fatty acid determination

Fatty acids were determined as methyl esters after in-situ transesterification (Wychen *et al*. 2013; Szpyrka *et al*. 2020), 20 mg of leaves (dry weight) was placed into a 2 ml chromatographic vial, then 25 µl of internal standard C15:0 (1000 µg · ml^{-1}), 200 µl of dichloromethane: methanol (2:1, v/v) and 300 µl of 0.6 M HCl in methanol were added to each sample. The internal standard C15:0 tripentadecanoin was purchased from Merck KGaA (Darmstadt, Germany). The standard of fatty acids: 37 Supelco component FAME MIX CRM47885 (Merck KGaA, Darmstadt, Germany) was used for quantification. The vials were sealed with PTFE caps, the contents of the vial were shaken by hand for 1 minute and oven heated at 85°C ± 3°C for 1 hour. The vials were then cooled (15 minutes) to room temperature. After cooling, 1 ml of petroleum ether was added to the vial, and the vial was shaken by hand for 1 minute and allowed to separate for 1 hour. The upper phase (100 µl) was transferred to a 2 ml chromatographic vial and 400 µl of petroleum ether was added. The determination was carried out using a gas chromatograph (Agilent Technologies, model 7890A, Palo Alto, CA, USA) with a mass detector (Agilent Technologies, model 7000, Palo Alto, CA, USA) in SIM mode (Single Ion Monitoring), with the following set-up: source temperature 230°C, ionization type – electron (EI), temperature program: 40–260°C, column HP–5 MS (Ultra Inert/ 30 m × 0.25 mm I.D. × 0.25-µm).

Statistical analysis

Population size of aphids, differences in the levels of FAs and their groups (saturated, monounsaturated and polyunsaturated) between the different treatments were analyzed using ANOVA. Post hoc Tukey's test was used where significant differences occurred. A Principal Component Analysis (PCA) was used to examine the effect of the applied treatments (herbicide, aphids and their interaction) on the FA profiles in hemp leaves. All statistical analyses were done using Statistica version 13 program (TIBCO Software Inc., 2017 (http://statistica.io; accessed on 22 October 2022) and PAST 4.0 software (Øyvind Hammer, Natural History Museum, University of Oslo, Oslo, Norway) (Hammer 2002).

Results

The population of cannabis aphids grew rapidly on hemp plants, especially those treated with quizalofop-P-tefuryl based herbicide, with a clear trend for more nymphs produced on plants treated with the herbicide. However, a statistically significant difference was found only on day 3 of the experimen t (ANOVA $F_{(1,10)} = 1013.7$; $p \leq 0.05$) (Fig. 3).

The initial concentration of quizalofop-P-tefuryl in *C. sativa* leaves was 0.037 mg · kg^{-1}. Ten days after application the amount had decreased to 0.006 mg · kg^{-1} (16% of the original concentration) (Fig. 4). The active substance dissipated according to the first order kinetic equation $y = 0.0393e^{-0.198x}$ (correlation coefficient $R = 0.948$) with a half-life of 3.5 days. On leaves with aphid herbivory, the active substance disappeared

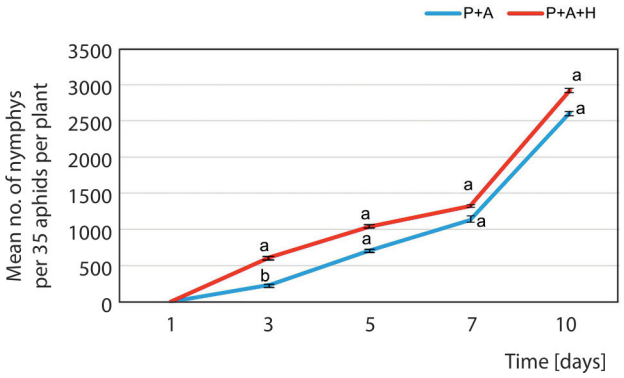

Fig. 3. Cannabis aphid (*Phorodon cannabis*) population development expressed as the mean number of nymphs produced by 35 females on untreated plants of *Cannabis sativa* (P+A) and plants treated with herbicide quizalofop-P-tefuryl (P+A+H). Line graphs with different letters represent significant differences at $p \leq 0.05$ (Tukey's test). Bars represent Standard Deviation. Graph shows the results of Experiment 1

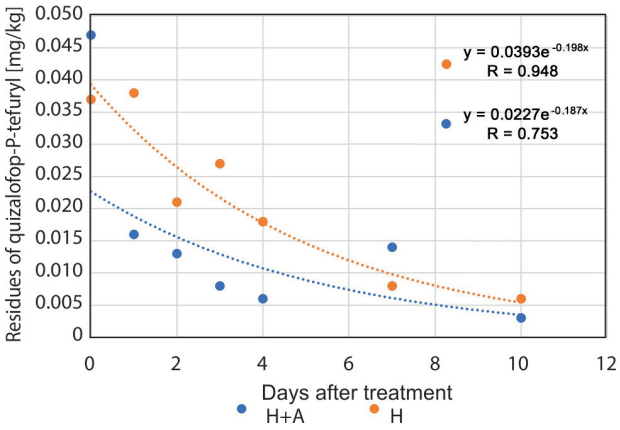

Fig. 4. The kinetics of quizalofop-P-tefuryl residues in hemp (*Cannabis sativa*) leaves treated with this herbicide and either infested with cannabis aphid (*Phorodon cannabis*) (H+A) or uninfested control (H)

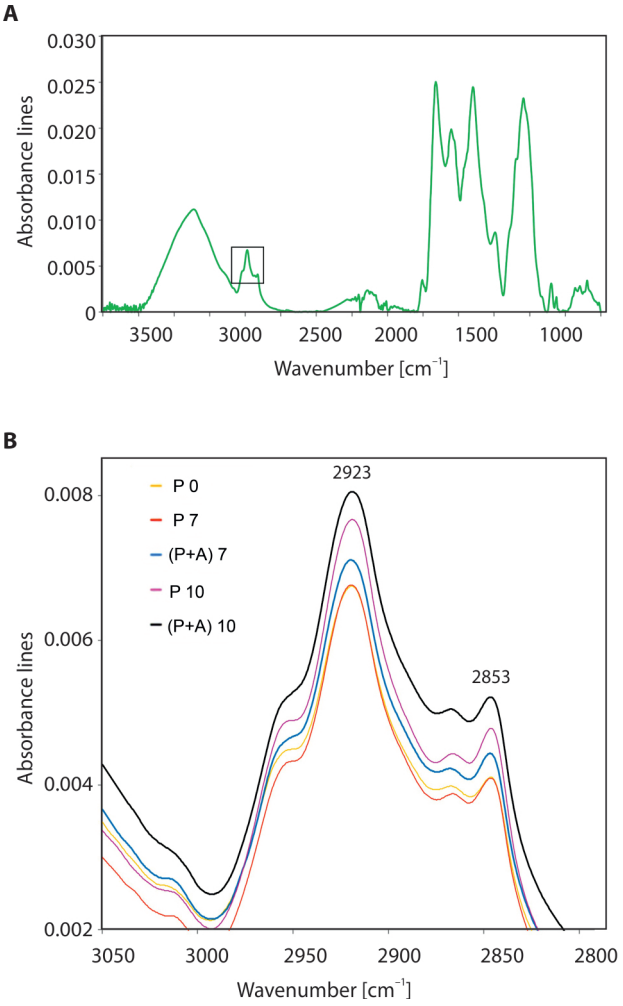

Fig. 5. Average Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectroscopy of hemp (*Cannabis sativa*). A – whole control spectrum (obtained from samples collected at the beginning of the experiment) with, spectral region connected with lipids and fatty acids framed by a black square; B – the bands from the spectral region marked by a black square in A, obtained for the different variants of the experiment. P 0 – hemp at the beginning of the experiment (control), P 7 – hemp after 7 days, (P+A) 7 – hemp infested with aphids after 7 days, P 10 – hemp after 10 days, (P+A) 10 – hemp infested with aphids after 10 days

according to equation $y = 0.0227e^{-0.187x}$ ($R = 0.753$) with the half-life of 3.7 days. No statistical differences between these two kinetics of residue dissipation were found.

Based on the ATR-FTIR spectroscopy method, differences between the maximum absorbance values from the spectral region related to lipids were detected (Fig. 5). These differences concerned peaks at wavenumbers 2923 and 2853 cm^{-1} corresponding to C–H stretching vibrations from lipids and fatty acids. In the ATR-FTIR spectra of samples from plants infested by aphids, peaks were significantly higher than untreated controls, especially after 10 days of their feeding activity (Fig. 5).

There were 21 fatty acids detected in hemp leaves, the proportions of which changed with the variant (herbicide, aphid infestation) (Tab. 1). The application of the herbicide did not affect the composition or ratio of fatty acids in hemp leaves, the fatty acid profile in the samples treated with the herbicide (P+H) did not differ significantly from the control plants (P). Proportions of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were similar between these treatments, and amounted to approximately 20%, 5% and 75% of the fatty acid profile, respectively. In the plants treated with the aphids and herbicide (P+A+H), a significant increase in the total profile of saturated ($F_{(1.8)} = 3567.6$; $p \leq 0.001$) and monounsaturated ($F_{(1.8)} = 2222.1$; $p \leq 0.001$) FAs were found as well as a significant decrease in the amount of polyunsaturated FAs ($F_{(1.8)} = 5867.8$; $p \leq 0.001$) on day 10 of the experiment (Fig. 6).

Considering the above mentioned increase in the concentration of SAFAs in hemp leaves, a significant increase was observed specifically for myristic acid, C14:0 ($F_{(1.24)} = 3197.7$; $p \leq 0.05$)(Fig. 7). Even though there was no statistical significance in the level of all MUFAs, a significant increase in the production of oleic acid (C18:1) was found ($F_{(1.24)} = 1532.5$; $p \leq 0.05$); the oleic acid content in hemp plants with the aphids and herbicide (P+A+H) increased 2.5-fold after 10 days (Fig. 8), while in the leaves treated only with aphids (P+A) or only with the herbicide (P+H) such an increase was not observed. There was also a significant decrease in the concentration of PUFAs with α-linolenic acid (ALA, C18:3) in particular. The decrease was observed while the hemp leaves were treated with aphids (P+A), but it was significantly higher when aphids fed on hemp plants treated with the herbicide (P+A+H) (Tab. 2).

Principal component analysis (PCA) showed that the profile of FAs in the herbicide treated plants (P+H) was comparable to control plants. The first PCA axis explained 91.6% of the variation, with high probability of sample separation due to plant feeding by aphids (Fig. 9). Plants infested by aphids (P+A) as well as those treated with herbicide and infested with aphids

Table 1. The profile of fatty acids in hemp leaf tissues (%)

Symbol of FA	IUPAC names	Common names	Time 0 (mean)		Time 24–240 h (mean)			
			P	P+H	P	P+H	P+A	P+A+H
C6:0	hexanoic acid	caproic acid	0.44	0.4	0.41	0.46	0.61	0.52
C14:0	tetradecenoic acid	**myristic acid**	0.13	0.03	0.11	0.13	0.91*	1.17*
C16:1	(Z)-hexadec-9-enoic acid	palmitoleic acid	1.44	1.5	1.47	1.47	1.52	1.55
C16:0	hexadecanoic acid	palmitic acid	13.84	13.45	14.01	13.79	13.67	13.89
C17:0	heptadecanoic acid	margaric acid, margarinic acid	0.22	0.21	0.22	0.23	0.25	0.22
C18:3 omega 6 GLA	(6Z,9Z,12Z)-octadeca-6,9,12-trienoic acid	all-cis-6,9,12-octadecatrienoic acid, gamma-linolenic acid, gamolenic acid	1.08	0.97	0.99	1.16	1.06	1.13
C18:2 omega 6	(9Z,12Z)-octadeca-9,12-dienoic acid	linoleic acid, telfairic acid	10.44	11.73	10.07	10.09	9.28	10.56
C18:1 omega 9	(Z)-octadec-9-enoic acid	**oleic acid**, cis-9-octadecenoic, elaidoic	4.56	6.89	4.09	3.85	4.71*	5.41*
C18:3 omega 3 ALA	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	**alpha-linolenic acid** cis,cis,cis-9,12,15-octadecatrienoate	61.61	58.6	62.29	62.38	61.60	59.00*
C18:0	octadecanoic acid	stearic acid	1.72	1.82	1.73	1.60	1.78	1.80
C20:5 omega 3 EPA	(5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic acid	cis-5,8,11,14,17-eicosapentaenoic acid, timnodonic acid, EPA	0.14	0.15	0.13	0.13	0.11	0.14
C20:3 omega 6	(8Z,11Z,14Z)-icosa-8,11,14-trienoic acid	cis,cis,cis-8,11,14-eicosatrienoic acid, dihomo-gamma-linolenic acid, DGLA	0.21	0.21	0.20	0.21	0.18	0.23
C20:2	(11Z,14Z)-icosa-11,14-dienoic acid	cis-11,14-eicosadienoic acid, eicosadienoic acid	0.27	0.27	0.26	0.26	0.26	0.28
C20:1	(Z)-icos-11-enoic acid	cis-11-eicosenoic acid, gondoic acid	0.19	0.22	0.19	0.19	0.17	0.21
C20:3 omega 3	(11Z,14E,17E)-icosa-11,14,17-trienoic acid	cis-11,14,17-eicosatrienoic	0.24	0.22	0.25	0.25	0.23	0.25
C20:0	icosanoic acid	arachidic acid, eicosanoic acid	0.98	0.93	1.07	1.12	1.14	1.14
C21:0	heneicosylic acid	heneicosylic acid	0.19	0.19	0.19	0.19	0.18	0.19
C22:1	(*Z*)-docos-13-enoic acid	cis-13-docosenoic acid, erucic acid	0.17	0.18	0.16	0.16	0.16	0.18
C22:0	docosanoic acid	behenic acid	1.04	1	1.09	1.16	1.13	1.06
C23:0	tricosanoic acid	tricosylic acid	0.19	0.18	0.19	0.19	0.18	0.19
C24:0	tetracosanoic acid	lignoceric acid	0.84	0.8	0.87	0.98	0.85	0.90

* significant differences between the different treatments for each acid, $p \leq 0.05$, Tukey's test was used

Gray highlight and bold letters indicate the statistical differences in fatty acid content.

Time 0 (mean) – mean fatty acid content (%) in hemp (*Cannabis sativa*) leaves at the beginning of the experiment, 24–240 h (min-max) – the lowest and the highest values in 24-240 h of the experiment; fatty acid content (%) in leaves of untreated plants (P), plants treated with the herbicide containing quizalofop-P-tefuryl (P+H), plants with aphid infestation (P+A), plants treated with herbicide and with aphid infestation (P+A+H)

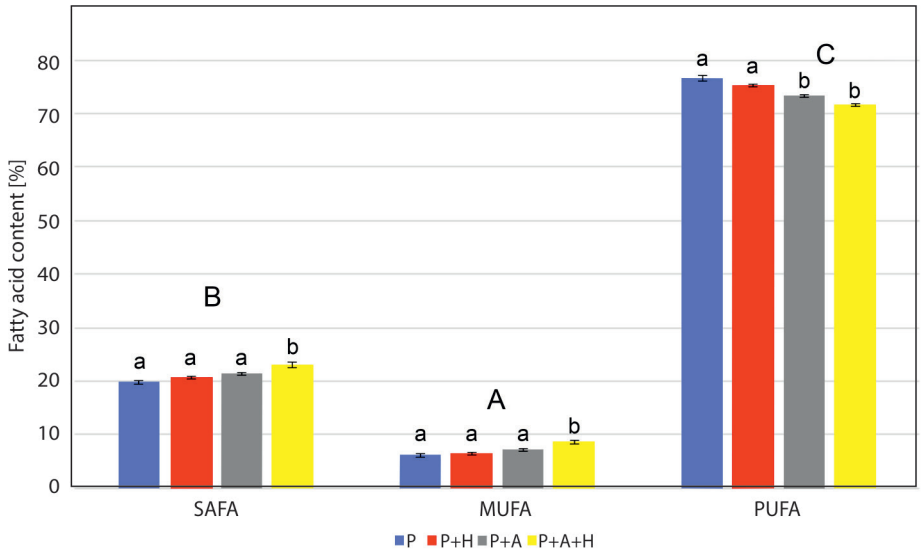

Fig. 6. The proportion of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in hemp (*Cannabis sativa*) leaves of untreated plants (P), plants treated with the herbicide containing quizalofop-P-tefuryl (P+H), plants with aphid infestation (P+A), plants treated with herbicide and with aphid infestation (P+A+H). Capital letters in the statistical analysis refer to the results of Tukey's test between treatments, lower case letters refer to Tukey's test between days. Bars with different letters differ significantly at $p < 0.05$

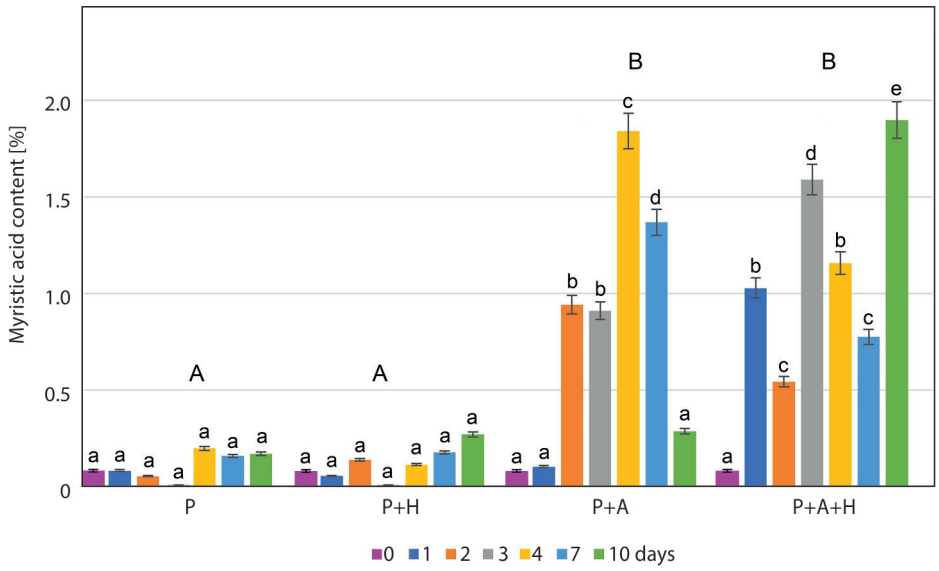

Fig. 7. The percent of myristic acid in hemp (*Cannabis sativa*) leaves of untreated plants (P), plants treated with the herbicide containing quizalofop–P–tefuryl (P+H), plants with aphid infestation (P+A), plants treated with the herbicide and with aphid infestation (P+A+H) at different intervals of the experiment. Capital letters in the statistical analysis refer to the results of Tukey's test between treatments, lower case letters refer to Tukey's test between days. Bars with different letters differ significantly at $p < 0.05$

(P+A+H) significantly differed from the control samples due to the greater content of oleic acid and myristic acid.

Discussion

The study confirmed the hypothesis that foraging of cannabis aphids as well as plant protection compounds affect the composition of fatty acids in hemp leaves. Based on ATR-FTIR analysis of FA composition in hemp leaves infested by aphids, followed by GC-MS analysis, three important, statistically significant findings concerning the fatty acid composition of hemp plants were found: 1) an increase of SAFA mainly due to myristic acid (7-fold for P+A and 9-fold for P+A+H); 2) an increase of oleic acid, which belongs to MUFA (2.5-fold); 3) a decrease of PUFA, primarily α-linolenic (ALA), in aphid infested as well as herbicide

Table 2. Profile (%) of myristic acid (C14:0), oleic acid (C18:1) and α-linolenic acid (C18:3) in hemp leaf tissues at different time-points of the experiment

Symbol of FA	Time 24 h				Time 48 h				Time 72 h			
	P	P+H	P+A	P+A+H	P	P+H	P+A	P+A+H	P	P+H	P+A	P+A+H
C14:0	0.08	0.06	0.10	1.03	0.05	0.14	0.94	0.54	0.00	0.01	0.91	1.59
C18:1 omega 9	3.71	3.46	5.00	3.56	3.57	4.12	3.84	5.53	4.82	3.98	4.69	3.91
C18:3 omega 3 ALA	61.74	61.00	60.88	59.29	61.78	60.59	62.38	58.76	63.08	61.97	61.33	58.14
Symbol of FA	Time 96 h				Time 7 days				Time 10 days			
	P	P+H	P+A	P+A+H	P	P+H	P+A	P+A+H	P	P+H	P+A	P+A+H
C14:0	0.20	0.11	1.84	1.16	0.16	0.18	1.37	0.78	0.17	0.27	0.29	1.90
C18:1 omega 9	4.93	4.76	5.25	5.49	3.35	3.23	4.55	3.58	4.19	3.56	4.94	10.36
C18:3 omega 3 ALA	64.45	64.17	60.87	58.79	62.30	64.30	61.96	62.29	60.37	62.24	62.20	56.72

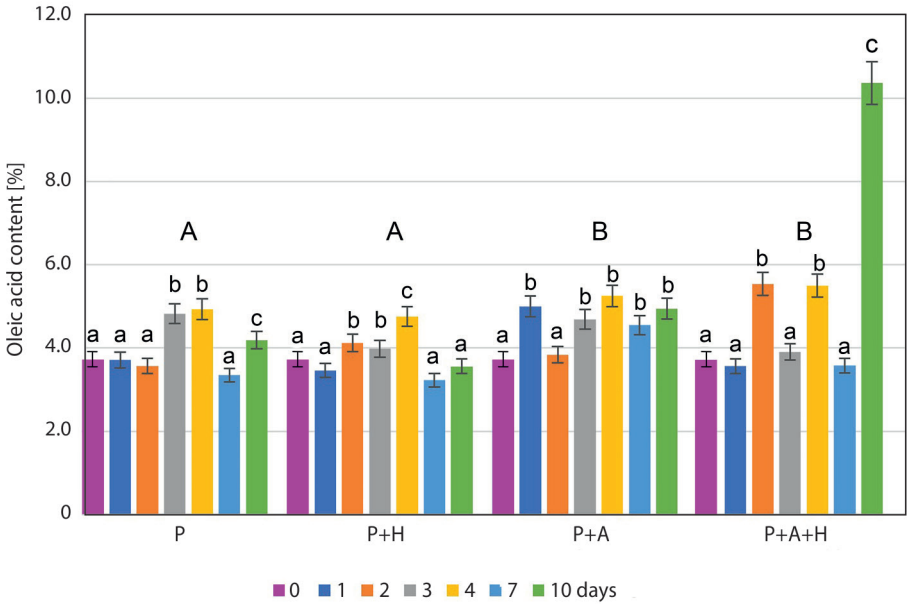

Fig. 8. The percent of oleic acid in hemp (*Cannabis sativa*) leaves of untreated plants (P), plants treated with the herbicide containing quizalofop-P-tefuryl (P+H), plants with aphid infestation (P+A), plants treated with the herbicide and with aphid infestation (P+A+H) on subsequent days of the experiment. Capital letters in the statistical analysis refer to the results of Tukey's test between treatments, lower case letters refer to Tukey's test between days. Bars with different letters differ significantly at $p < 0.05$

treated and aphid infested plants. The connection between ALA and the jasmonate pathway (Blée 2002; Prost 2005; Lim *et al.* 2017; He *et al.* 2018) supports the idea that aphid populations could manipulate the jasmonate pathway in plants.

An increase in the aphid population, caused by quizalofop-P-tefuryl via fatty acid reprogramming was also hypothesized. The decrease of ALA, being the precursor of jasmonic acid, could reduce the defense power of hemp plants and lead to an increase of the cannabis aphid population. Alternatively, the impact of myristic acid or the decrease of ALA alone could have been the only cause of the observed effect. Difficulties in obtaining permission for further studies on hemp made it impossible to verify these well-grounded speculations.

The ATR-FTIR vibrational spectroscopy technique used in this study was an effective tool to investigate changes in fatty acid content in oil hemp leaves at the primary phase of this study. This spectroscopic technique provides spectral bands that are molecule specific and give direct details about the biochemical composition of the studied object. The analysis is quick, easy, and requires little sample material with minimal sample preparation (Smith 2011). The use of the ATR-FTIR method proved useful for rapid screening of fatty acid profiles in the studied samples. In the present study this technique gave the first indication

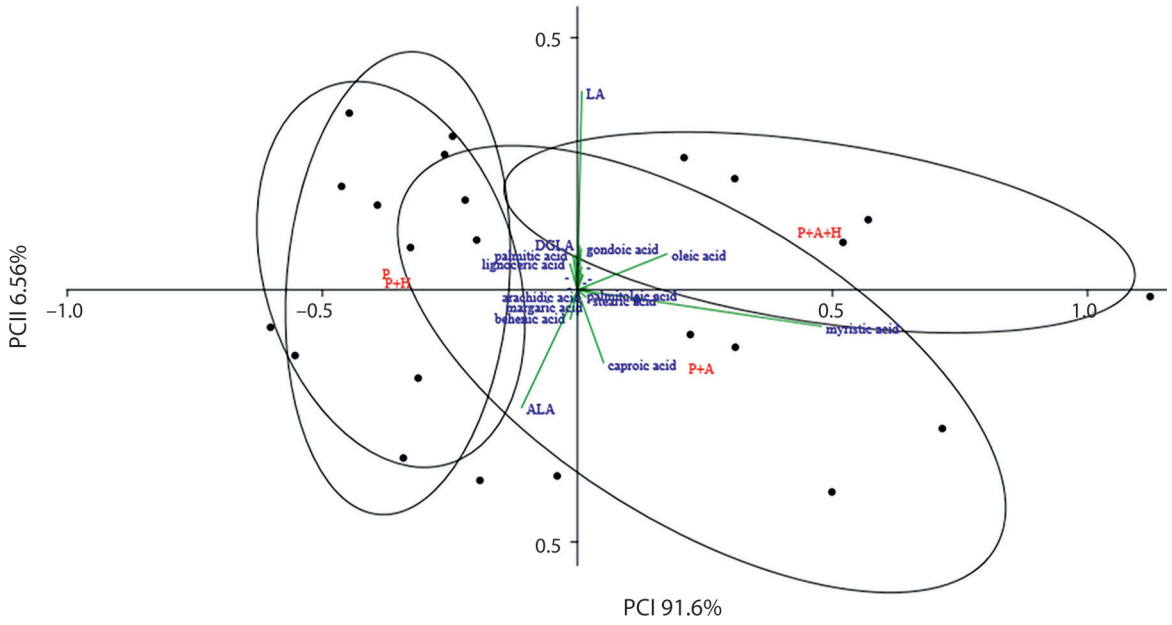

Fig. 9. Principal component analysis (PCA) of fatty acid profile in hemp (*Cannabis sativa*) leaves

of substantial changes in plant FA content and composition caused by aphids, which was further proved by gas chromatography. Clear differences between plants with and without aphids at 7 and 10 days of investigation were observed using both techniques.

Myristic acid shows highly important but various impacts on insects. It was found to attract mosquitos (Mathew *et al.* 2013) and some studies demonstrated its beneficial effect on insect pests (Bergman *et al.* 1991). Impairing fatty acid synthesis in pea aphids prolonged the nymphal growth period and decreased the aphid body weight (Zhou *et al.* 2021). Conversely, supplementation of myristic acid to these aphids restored their normal development and weight gain. However, in this study an increased size of aphids feeding on cannabis with highly increased myristic acid was not observed. In contrast, other studies have demonstrated a larvicidal and repellent effect of myristic acid against various insects (Farag *et al.* 2011; Sivakumar *et al.* 2011). Da Silva and Ricci-Junior (2020) reported the successful use of the polymeric controlled release/mixing system of saturated and unsaturated fatty acids for slow release mosquito repellents. Interestingly, this controlled-release of the natural plant mosquito repellent patented by Huizhi and Yubo (2017) involved an encapsulation in myristic and linoleic acids and was effective for 10–12 days with efficiency exceeding 90%. Insecticidal compounds found in *Ricinus communis* L. controlling the sugarcane aphid, *Melanaphis sacchari* Zehntner contained myristic and stearic acids in its most detrimental fraction (Sotelo-Leyva *et al.* 2020), with stearic acid having a stronger effect (Aguilar-Marcelino *et al.* 2022). Contact toxicity activities of lauric, myristic and palmitic acids towards *Sitophilus granarius* L. showed the highest mortality rate (53.34%) of myristic acid among the tested pure fatty acids, with much lower activities of palmitic and lauric acids equal to 17.75% and 4.32%, respectively (Abay *et al.* 2013). This adverse activity of myristic acid could explain why the sudden increase of the aphid population, observed on day 3 of the experiment, gradually stopped when myristic acid was produced by plants in such excess. The number of aphids feeding on cannabis plants treated with the herbicide remained high, but the effect was no longer significant.

The involvement of fatty acids in pathogen and herbivore defense against aphids has been previously demonstrated (Farmer *et al.* 2003; Lim 2017), especially in the context of phytohormonal signaling in response to aphid feeding (Morkunas *et al.* 2011). Cannabis aphid feeding significantly increased levels of major phytohormones such as jasmonic, salicylic and abscisic acids, that play major roles in plant defense responses against aphid species (MacWilliams *et al.* 2023). Moreover, the interplay between phytohormone pathways and cannabinoid synthesis was demonstrated, with cannabidiol (CBD) playing an adaptive role in this complex process. Linolenic acid belonging to PUFAs, the precursor of jasmonic acid, one of the most studied oxylipins, promoted the accumulation of metabolites that were detrimental to green peach aphid *Myzus persicae* (Louis and Shah 2013). However, oxylipins consumed by the aphid from the plant changed gene expression and the physiology of insects, leading them to overcome or bypass plant defense for successful herbivory (Lim *et al.* 2017). Additionally, an experiment with a series of mutants of *Arabidopsis thaliana* showed that oxylipins facilitate infestation of shoots

by green peach aphids (Nalam *et al*. 2012). Soybean aphids (*Aphis glycines*) avoided effective defenses by inhibition of jasmonate–regulated plant responses; aphid infestation reduced levels of PUFAs in leaves with a concomitant increase in palmitic acid, SAFA (Kanobe *et al*. 2015).

The mechanism of plant defense is similar in different aphid-crop plant systems (Wu and Baldwin 2010; Pitino and Hogenhout 2013; Nalam *et al*. 2018; Kumar 2020). The present study supports the idea that aphids block jasmonate-dependent plant defense by reduction of α-linolenic acid which serves as its precursor (Kanobe *et al*. 2015). This clever mode of action enabling the aphids to inhibit the induction of effective defenses in plants was termed 'metabolic hijacking'. Based on the changes in fatty acid levels, Kanobe *et al* (2015) hypothesized that aphids potentially induce interference in the fatty acid desaturation pathway by fatty acid desaturases (FADs), likely reducing FAD2 and FAD6 activity that leads to a reduction in polyunsaturated fatty acids, thus blocking JA-dependent defenses. Similarly, we presume that cannabis aphids also block the JA-dependent defense of hemp plants by reducing the hormone precursor, α-linolenic acid (ALA). Interestingly, recent studies also demonstrated that certain FADs were important susceptibility factors in plant-aphid interactions and showed that aphid resistance was more strongly associated with differences in saturated FAs (Li *et al.* 2021). In this study oleic acid C18:1 nearly tripled as a result of aphid feeding. A similar defensive response was observed in *M. persicae* (Louis *et al*. 2010).

The effects of fatty acids and their methyl esters are not confined to aphids; they are a common phenomenon in plant-insect interactions. McFarlane and Henneberry (1965) reported the inhibition of growth of the cricket, *Gryllodes sigillatus* by SAFAs. In their study a high level of lauric acid (1%) inhibited growth of the cricket through the alimentary tract. The effective fatty acids were lauric, myristic, stearic, and behenic as well as methyl esters of palmitic, myristic, stearic, and oleic acids. The study of two columnar cacti in the Sonoran Desert revealed the presence of medium chain fatty acids in their necrotic tissues, serving as feeding and breeding substrates for *Drosophila mojavensis*. The strongest effect was attributed to caprylic acid however, the inhibitory effect of myristic acid on *Drosophila* larvae development was also reported (Fogleman and Kircher 1986). The effect of FAs on reproduction of the hide beetle *Demestes maculatus* revealed that short chain C5-C10 FAs also suppress fertility of the insect when added to the diet of females, but they do not affect males (Cohen and Levinson 1972). Short chain FAs were incorporated into the egg lipids and interfered with embryonic development. In contrast, saturated and unsaturated homologs of lauric acid increased egg production. The density-independent diapause was attributed to the lipids in insect diet (Nair and Desai 1972).

The interplay between FAs in plants and insects is a fascinating field of study. The novel techniques of gas chromatography coupled with very sensitive mass spectrometry detectors and rich bioinformatic databases make these studies increasingly possible and refined. Novel tools also offer the possibility of manipulation of biosynthetic pathways in transgenic plants to redesign plant metabolism toward the production of specific compounds (Ohlrogge 1994). Plant oil composition can be substantially altered by either turning on or off the expression of a single enzyme activity (Zhao *et al.* 2021; He *et al.* 2020; Liao *et al.* 2022; Chao *et al.* 2023). Apparently, the skill of altering fatty acid composition is also naturally achieved by insects, including the cannabis aphid *P. cannabis*.

C18 unsaturated FAs play important roles in plant defense against various biotic and abiotic stresses (He and Ding 2020). Nevertheless, changes in fatty acid composition and their proportions in plant cells and phloem require time. Based on the reaction observed in Experiment 1, where the trend in favor of aphid feeding on herbicide treated plants started on day 1 and continued until the last day of the experiment (day 10), with significant differences found on day 3, it is clear that the process takes days rather than hours or minutes. The differences in SAFA, MUFA and PUFA concentrations were significantly higher when plants were treated with the herbicide quizalofop-P-tefuryl. The previous study also showed that the herbicide presence in phloem sap coincided with higher fecundity of cannabis aphids (Durak *et al.* 2021).

The highest aphid population on day 3 after herbicide application matches the residue kinetics of quizalofop-P-tefuryl. In the present study the half-life of this compound was 3.5 days in leaves of herbicide-treated plants and 3.7 days in leaves of plants treated with the herbicide and infested with aphids. The decrease of quizalofop-P-tefuryl in leaves coincided with the increase of aphid fecundity on day 3. On days 5, 7 and 10 it was still higher in herbicide-treated plants but the difference between herbicide treated and untreated plants was statistically insignificant.

It was demonstrated that the application of herbicide containing quizalofop-P-tefuryl did not affect the fatty acid desaturation pathway in hemp leaves. The compound belongs to the class of aryloxyphenoxypropionic herbicides (commonly called 'FOPs') such as diclofop-P and fluazifop-P, which are taken up *via* leaves and hinder the *de novo* synthesis of fatty acids by inhibition of the enzyme Acetyl-CoA carboxylase (ACCase) (EFSA 2008). This effect, however, is restricted to monocotyledons, so dicotyledonous plants, like hemp, should not exhibit this effect. Quizalofop-P-

-tefuryl is non-persistent in the environment, having soil DT90 values ranging from 0.30 to 1.16 days (Ohlrogge 1994). Quizalofop-P0tefuryl DT50 in plant matrix was in the range 0.7–5.6 days. The half-lives on black gram (*Vigna mungo*) were 0.47 and 0.64 days after application of 40 g · ha^{-1} and 80 g · ha^{-1} of active substance, respectively (Mukhopadhyay *et al.* 2012). Some of the herbicide fluid was taken up by the aphids with the plant sap, changing the usual composition of their diet and causing stress. As a result of the threat, the fecundity of aphids was raised and their population increased, which resulted in stronger defensive reactions of plants.

Conclusions

Quizalofop-P-tefuryl rapidly degraded in leaves and was non-persistent in hemp plants. The application of a herbicide containing this compound did not affect the fatty acid desaturation pathway, suggesting it can be safely used as a graminicide to control grasses in hemp crops. However, *P. cannabis* aphids feeding on hemp plants treated with this herbicide increased their population. The statistical significance of this phenomenon was demonstrated on day 3 after herbicide treatment. Reprogramming of the concentrations of FAs was observed; the significant increase in myristic acid (C14:0), belonging to saturated fatty acids and oleic acid (C18:1) being a monounsaturated fatty acid was detected, with 7- to 9-fold and 2.5-fold increases, respectively. This effect is attributed to the defense reaction of plants subjected to strong biotic stress caused by aphid feeding. A significant decrease of polyunsaturated fatty acid, primarily α-linolenic acid, which acts as the precursor in the jasmonate signaling pathway, was possibly caused by aphids to suppress the defense mechanism of plants in their favor.

The study revealed that non-target plant protection products can significantly increase insect pest population. Indication of such treatments and compounds is crucial for improving pest management strategies. Aphid feeding and quizalofop-P-tefuryl application strongly affected the FAs composition in hemp leaves especially in interaction with the herbicide, which showed that some effects can be detected solely by complex investigation of several jointly operating factors. Multifactorial studies can greatly help in better understanding the phenomena observed in agricultural fields treated with plant protection products.

Acknowledgements

The authors would like to thank Paulina Książek-Trela, Magdalena Wlaszczyk and Katarzyna Beczek for their valuable technical help.

References

Abay G., Altun M., Karakoç Ö.C., Gül F., Demirtas I. 2013. Insecticidal activity of fatty acid-rich Turkish bryophyte extracts against *Sitophilus granarius* (Coleoptera: Curculionidae). Combinatorial Chemistry & High Throughput Screening 16 (10): 806–816. DOI: https://doi.org/10.2174/13862073113169990049

Aguilar-Marcelino L., Pineda-Alegría J.A., Salinas-Sánchez D.O., Hernández-Velázquez V.M., Silva-Aguayo G.I., Navarro-Tito N., Sotelo-Leyva C. 2022. *In vitro* insecticidal effect of commercial fatty acids, β-sitosterol, and rutin against the sugarcane aphid, *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae). Journal of Food Protection 85 (4): 671–675. DOI: https://doi.org/10.4315/JFP-21-329

Bakro F., Jędryczka M., Wielgusz K., Sgorbini B., Inchingolo R., Cardenia V. 2020. Simultaneous determination of terpenes and cannabidiol in hemp (*Cannabis sativa* L.) by fast gas chromatography with flame ionization detection. Journal of Separation Science 43 (14): 2817–2826. DOI: http://doi.org/10.1002/jssc.201900822

Bakro F., Wielgusz K., Bunalski M. Jędryczka M. 2018. An overview of pathogen and insect threats to fibre and oilseed chemp (*Cannabis sativa* L.) and methods for their biocontrol. Integrated Control in Oilseed Crops IOBC-WPRS Bulletin 136: 9–20. https://www.cabidigitallibrary.org/doi/full/10.5555/20203512322

Blackman R.L., Eastop V.F. 2024. Aphids of the World's Plants: An Online Identification and Information Guide. [Available from: http://www.aphidsonworldsplants.info] [Accessed 15 May 2024].

Blée E. 2002. Impact of phyto-oxylipins in plant defense. Trends of Plant Science 7: 315–322. DOI: https://doi.org/10.1016/S1360-1385(02)02290-2

Bergman D.K., Dillwith J.W., Berberet R.C. 1991. Spotted alfalfa aphid, *Therioaphis maculata*, fatty acids relative to the condition and susceptibility of its host. *Archives* of *Insect Biochemistry* and *Physiology* 18: 1–12. DOI: https://doi.org/10.1002/arch.940180102

Bos J.I.B., Prince D., Pitino M., Maffei M.E., Win J., Hogenhout S.A. (2010) A Functional Genomics Approach Identifies Candidate Effectors from the Aphid Species *Myzus persicae* (Green Peach Aphid). PLoS Genetics 6 (11): e1001216. DOI: https://doi.org/10.1371/journal.pgen.1001216

Bromilow R., Chamberlain K., Evans A. 1990. Physicochemical aspects of phloem translocation of herbicides. Weed Science 38 (3): 305–314. DOI: https://doi.org/10.1017/S0043174500056575

Callaway J.C. 2004. Hempseed as a nutritional resource – an overview. Euphytica 140 (1–2): 65–72. DOI: http://doi.org/10.1007/s10681-004-4811-6

Cantele C., Bertolino M., Bakro F., Giordano M., Jędryczka M., Cardenia V. 2020. Antioxidant effects of hemp (*Cannabis sativa* L.) inflorescence extract in stripped linseed oil. Antioxidants 9 (11): 1131. DOI: https://doi.org/10.3390/antiox9111131

Chao H., Yu-Ting L., Yu-Xi L., Ge-Fei H., Xue-Qing Y. 2023. Molecular interaction network of plant-herbivorous insects. Advanced Agrochemistry 3 (1): 74–82. DOI: https://doi.org/10.1016/j.aac.2023.08.008

Christensen S.A., Kolomiets M.V. 2011. The lipid language of plant-fungal interactions. Fungal Genetics and Biology 48: 4–14. DOI: http://doi.org/10.1016/j.fgb.2010.05.005

Cohen E., Levinson Z. 1972. The effect of fatty acids on reproduction of the hide beetle *Dermestes maculatus* (Dermestidae; Coleoptera). Life Sciences 11: 293–299. DOI: http://10.1016/0024-3205(72)90082-3

Cornelissen J.H.C., Lavorel S., Garnier E., Díaz S., Buchmann N., Gurvich D.E., Reich P.B., ter Steege H., Morgan H.D., van der Heijden M.G.A., Pausas J.G., Poorter H. 2003. A handbook of protocols for standardized and easy

measurement of plant functional traits worldwide. Australian Journal of Botany 51: 335–380. DOI: http://dx.doi.org/10.1071/BT02124

Cranshaw W., Halbert S., Favret C., Britt K. Miller G. 2018. *Phorodon cannabis* Passerini (Hemiptera: Aphididae), a newly recognized pest in North America found on industrial cannabis. Insecta Mundi 662: 1–12. DOI: http://doi.org/10.5281/zenodo.3710022

da Silva M.R.M., Ricci-Júnior E. 2020. An approach to natural insect repellent formulations: from basic research to technological development. Acta Tropica 2020 212: 105419. DOI: http://doi.org/10.1016/j.actatropica.2020.105419

de Vos M., Kim J.H., Jander G. 2007. Biochemistry and molecular biology of Arabidopsis-aphid interactions. Bioassays 9 (9): 871–83. DOI: http://doi.org/10.1002/bies.20624

Dobermann D., Field L.M., Michaelson L.V. 2019. Impact of heat processing on the nutritional content of *Gryllus bimaculatus* (black cricket). Nutrition Bulletin 2: 116–122. DOI: https://doi.org/10.1111/nbu.12374

Document N° SANTE 11312/2021. 2021. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. European Commission Directorate-General for Health and Food Safety. [Available from: https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE_11312_2021.pdf] [Accessed 25 January 2024]

Durak R., Jedryczka M., Czajka B., Dampc J., Wielgusz K., Borowiak-Sobkowiak B. 2021. Mild Abiotic Stress Affects Development and Stimulates Hormesis of Hemp Aphid *Phorodon cannabis*. *Insects* 12 (5): 420. DOI: https://doi.org/10.3390/insects12050420

Elzinga D.A., De Vos M., Jander G. 2014. Suppression of plant defences by a *Myzus persicae* (green peach aphid) salivary effector protein. Molecular Plant Microbe Interactions 27 (7): 747–56. DOI: http://doi.org/10.1094/MPMI-01-14-0018-R

European Food Safety Authority. 2008. Conclusion on the Peer Review of Quizalofop-P. European Food Safety Authority Scientific Report 205: 1–216. [Available from: https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2009.205r] [Accessed 14 January 2024] DOI: https://doi.org/10.2903/j.efsa.2009.205r

Farag M., Ahmed M.H., Yousef H., Abdel-Rahman A.A. 2011. Repellent and insecticidal activities of *Melia azedarach* L. against cotton leafworm, *Spodoptera littoralis* (Boisd.). Zeitschrift für *Naturforschung* section C Journal of Biosciences 66 (3-4): 129–35. DOI: http://doi.org/10.1515/znc-2011-3-406

Farinon B., Molinari R., Costantini L., Merendino N. 2020. The seed of industrial hemp (*Cannabis sativa* L.): Nutritional Quality and Potential Functionality for Human Health and Nutrition. Nutrients 2 (7): 1935. DOI: http://doi.org/10.3390/nu12071935

Farmer E.E., Alméras E., Krishnamurthy V. 2003. Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. Current Opinion in Plant Biology 6 (4): 372–378. DOI: http://doi.org/10.1016/s1369-5266(03)00045-1

Fogleman J.C., Kircher H.W. 1986. Differential effects of fatty acid chain length on the viability of two species of cactophilic *Drosophila*. Comparative Biochemistry and Physiology – Part A: Physiology 83 (4): 761–764. DOI: https://doi.org/10.1016/0300-9629(86)90724-3

Golimowski W., Teleszko M., Marcinkowski D., Kmiecik D., Grygier A., Kwasnica A. 2022. Quality of oil pressed from hemp seed varieties: 'Earlina 8FC', 'Secuieni Jubileu' and 'Finola'. Molecules 27: 3171. DOI: https://doi.org/10.3390/molecules27103171

Hammer O., Harper D.A.T., Ryan P.D. 2002. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 1–9. DOI: http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Harwood J.L. 1988. Fatty Acid Metabolism. Annual Review of Plant Biology 39: 101–138. DOI: http://doi.org/10.1146/annurev-phyto-080516-035406

He M., Ding N.Z. 2020. Plant Unsaturated Fatty Acids: Multiple Roles in Stress Response. Frontiers in Plant Science 11: 562785. DOI: https://doi.org/10.3389/fpls.2020.562785

He M., He C.Q., Ding N.Z. 2018. Abiotic stresses: general defenses of land plants and chances for engineering multistress tolerance. Frontiers in Plant Science 9: 1771. DOI: https://doi.org/10.3389/fpls.2018.01771

He M., Qin Chun-Xue, Wang Xu, Ding Nai-Zheng. 2020. Plant Unsaturated Fatty Acids: Biosynthesis and Regulation. Frontiers in Plant Science. 11. DOI: http://doi.org/10.3389/fpls.2020.00390

Heie O.E. 1994. The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. V, Family Aphididae, Part 2 of Tribe Macrosiphini of Subfamily Aphidinae. 1st ed. Brill, Leiden, The Netherlands, 242 pp. DOI: https://archive.org/details/entomologisk-tidskrift-118-010

Huizhi L., Yubo Z. 2017. Preparation method of controlled--release natural plant mosquito repellent. CN Patent 102016000854902.

Juárez M.P., Napolitano R. 2000. Effects of organic acids on lipid synthesis and ecdysis in *Triatoma infestans* eggs. Comparative Biochemistry and Physiology part B: Biochemistry & Molecular Biology 125: 503–510. DOI: https://doi.org/10.1016/S0305-0491(00)00155-3

Kachroo P., Shanklin J., Shah J., Whittle E.J., Klessig D.F. 2001. A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proceedings of the National Academy of Sciences of the United States of America 98: 9448–9453. DOI: http://doi.org/10.1073/pnas.151258398

Kanobe C., McCarville M.T., O'Neal M.E., Tylka G.L., MacIntosh G.C. 2015. Soybean Aphid Infestation Induces Changes in Fatty Acid Metabolism in Soybean. PLoS ONE 10 (12): e0145660. DOI: http://doi.org/10.1371/journal.pone.0145660

Kim H.U. 2020. Lipid Metabolism in Plants. Plants 9 (7): 871. DOI: https://doi.org/10.3390/plants9070871

Kumar S. 2020. Aphid-Plant Interactions: Implications for pest management. plant communities and their environment. IntechOpen. DOI: http://doi.org/10.5772/intechopen.84302

Lemberkovics E., Veszki P., Verzarpetri G. Trka A. 1979. Contributions to the essential oil composition of the flowers and leaves of *Cannabis-sativa* L. Medicinal Plants of the World (Humana), vol. 3. Ed. Ivan A Ross, Humana Press, USA, 648 pp.

Leonard W., Zhang P., Ying D., Fang Z.X. 2020. Hempseed in food industry: Nutritional value, health benefits, and industrial applications. Comprehensive Reviews in Food Science and Food Safety *19* (1): 282–308. DOI: http://doi.org/10.1111/1541-4337.12517

Li J., Galla A., Avila C.A., Flattmann K., Vaughn K., Goggin F.L. 2021. Fatty acid desaturases in the chloroplast and endoplasmic reticulum promote susceptibility to the green peach aphid *Myzus persicae* in *Arabidopsis thaliana*. Molecular Plant Microbe Interactions 34 (6): 691–702. DOI: http://doi.org/10.1094/MPMI-12-20-0345-R

Liao P, Lechon T, Romsdahl T, Woodfield H, Fenyk S, Fawcett T, Wallington E., Bates R.E., Chye M.L., Chapman K.D. Harwood J.L, Scofield S. 2022. Transgenic manipulation of triacylglycerol biosynthetic enzymes in *B. napus* alters lipid-associated gene expression and lipid metabolism. Scientific Reports 12: 3352. DOI: https://doi.org/10.1038/s41598-022-07387-x

Lim G.H., Singhal R., Kachroo A., Kachroo P. 2017. Fatty acid- and lipid-mediated signaling in plant defense. Annual Review of Phytopathology 55: 505–536. DOI: http://doi.org/10.1146/annurev-phyto-080516-03540

Louis J., Leung Q., Pegadaraju V., Reese J., Shah J. 2010. PAD4-dependent antibiosis contributes to the ssi2-conferred hyper-resistance to the green peach aphid. Molecular Plant Microbe Interactions 23 (5): 618–627. DOI: http://doi.org/10.1094/MPMI-23-5-0618

Louis J., Shah J. 2013. *Arabidopsis thaliana – Myzus persicae* interaction: shaping the understanding of plant defense against phloem-feeding aphids. Frontiers in Plant Science 4: 213. DOI: https://doi.org/10.3389/fpls.2013.00213

Lupette J., Benning C. 2020. Human health benefits of very--long-chain polyunsaturated fatty acids from microalgae. Biochimie 178: 15–25. DOI: https://doi.org/10.1016/j.biochi.2020.04.022

MacWilliams J., Peirce E., Pitt W.J., Schreiner M., Matthews T., Yao L., Broeckling C., Nachappa P. 2023. Assessing the adaptive role of cannabidiol (CBD) in *Cannabis sativa* defense against cannabis aphids. Frontiers in Plant Science 14: 1223894. DOI: https://doi.org/10.3389/fpls.2023.1223894

Mathew N., Ayyanar E., Shanmugavelu S., Muthuswamy K. 2013. Mosquito attractant blends to trap host seeking *Aedes aegypti*. *Parasitology* Research 112 (3): 1305–12. DOI: https://doi.org/10.1007/s00436-012-3266-2

McCauley J.I., Meyer B.J., Winberg P.C., Skropeta D. 2016. Parameters affecting the analytical profile of fatty acids in the macroalgal genus Ulva. Food Chemistry 209: 332–340. DOI: http://doi.org/10.1016/j.foodchem.2016.04.039

McFarlane J.E., Henneberry G. O. 1965. Inhibition of the growth of an insect by fatty acids. Journal of Insect Physiology 11 (9): 1247–1252. DOI: https://doi.org/10.1016/0022-1910(65)90117-4

McPartland J.M., Clarke R.C., Watson D.P. 2000. Hemp Diseases and Pests Management and Biocontrol. 1st ed. CABI International, CABI Publishing: Wallingford, Oxon, UK, 251 pp.

Morkunas I., Mai V.C., Gabryś B. 2011. Phytohormonal signalling in plant responses to aphid feeding. Acta Physiologiae Plantarum 33: 2057–2073. DOI: http://doi.org/10.1007/s11738-011-0751-7

Mukhopadhyay S., Bhattacharyya A., Das S. 2012. Fate and persistence of herbicide quizalofop-p-tefuryl on black gram. Journal of Crop and Weed 8 (1): 190–192. DOI: https://www.cropandweed.com/vol8issue1/pdf2005/49.pdf

Nair K.S.S., Desai A.K. 1972. Some new findings on factors inducing diapause in *Trogoderma granarium* Everts (Coleoptera, Dermestidae). Journal of Stored Products Research 8: 27–54. DOI: https://doi.org/10.1016/0022-474X(72)90017-3

Nalam V., Louis J., Shah J. 2018. Plant defense against aphids, the pest extraordinaire. Plant Science 279: 96–107. DOI: https://doi.org/10.1016/j.plantsci.2018.04.027

Nalam V.J., Keeretaweep J., Sarowar S., Shah J. 2012. Root-derived oxylipins promote green peach aphid performance on *Arabidopsis* foliage. The Plant Cell 22 (4): 1643–1653. DOI: https://doi.org/10.1105/tpc.111.094110

Ohlrogge J.B. 1994. Design of new plant products: engineering of fatty acid metabolism. Plant Physiology 104: 821–826. DOI: http://doi.org/10.1104/pp.104.3.821

Pitino M., Hogenhout S.A. 2013. Aphid protein effectors promote aphid colonization in a plant species-specific manner. Molecular Plant Microbe Interactions 26 (1): 130–139. DOI: http://doi.org/10.1094/MPMI-07-12-0172-FI

Podbielska M., Kus-Liśkiewicz M., Jagusztyn B., Szpyrka E. 2023. Effect of microorganisms on degradation of fluopyram and tebuconazole in laboratory and field studies. Environmental Science and Pollution Research 30 (16): 1–15. DOI: https://doi.org/10.1007/s11356-023-25669-3.

Prost I., Dhondt S., Rothe G., Vicente J., Rodriguez M.J., Kift N. *et al*. 2005. Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. Plant Physiology 139: 1902–1913. DOI: http://doi.org/10.1104/pp.105.066274

Sivakumar R., Jebanesan A., Govindarajan M., Rajasekar P. 2011. Larvicidal and repellent activity of tetradecanoic acid against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say.) (Diptera: Culicidae). Asian Pacific Journal of Tropical Medicine 4 (9): 706–710. DOI: http://doi.org./10.1016/S1995-7645(11)60178-8

Słowik-Borowiec M., Szpyrka E., Książek-Trela P., Podbielska M. 2022. Simultaneous Determination of Multi-Class Pesticide Residues and PAHs in Plant Material and Soil Samples Using the Optimized QuEChERS Method and Tandem Mass Spectrometry Analysis. Molecules 27: 2140. DOI: https://doi.org/10.3390/molecules27072140

Smith B.C. 2011. Fundamentals of Fourier Transform Infrared Spectroscopy. 2nd ed. CRC Press; Taylor & Francis Group, Boca Raton, London, New York, 207 pp. DOI: https://doi.org/10.1201/b10777

Sotelo-Leyva C., Salinas-Sánchez D.O., Peña-Chora G., Trejo--Loyo A.G., González-Cortázar M., Zamilpa A. 2020. Insecticidal compounds in *Ricinus communis* L. (Euphorbiaceae) to control *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae). Florida Entomologist 103: 91–95. DOI: https://doi.org/10.1653/024.103.0415

Storelli M. 2014. Evaluation of toxic metal (Hg, Cd, Pb), polychlorinated biphenyl (PCBs), and pesticide (DDTs) levels in aromatic herbs collected in selected areas of Southern Italy. Environmental Science and Pollution Research 21: 946–953. DOI: http://doi.org/10.1007/s11356-013-1967-4

Szpyrka E., Broda D., Oklejewicz B., Podbielska M., Słowik--Borowiec M., Jagusztyn B., Chrzanowski G., Kus-Liskiewicz M., Duda M., Zuczek J., Wnuk M., Lewińska A. 2020. A Non-Vector Approach to Increase Lipid Levels in the Microalga *Planktochlorella nurekis*. Molecules 25 (2): 270. DOI: https://doi.org/10.3390/molecules25020270

Truta E., Surdu S., Rosu C.M., Asaftei M. 2009. Hemp -Biochemical diversity and multiple uses. Analele Stiintifice ale Universitatii Al. I Cuza din Iasi. Genetica si Biologie Moleculara 10: 1–8. [Available on: https://www.researchgate.net/profile/Elena-Truta/publication/236873721_Hemp_-Biochemical_diversity_and_multiple_uses/links/02e7e519c9b871cdd5000000/Hemp-Biochemical-diversity-and-multiple-uses.pdf]

Wu J., Baldwin IT. 2010. New insights into plant responses to the attack from insect herbivores. Annual Review of Genetics. 44:1–24. DOI: https://doi.org/10.1146/annurev-genet-102209-163500

Wychen S., Ramirez K., Laurens L.M.L. 2013. Determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by in situ Transesterification. Laboratory Analytical Procedure (LAP) DOI: https://doi.org/10.2172/1118085

Zhao Y., Cao P., Cui Y., Liu D., Li J., Zhao Y., Yang S., Zhang B., Zhou R., Sun M., Guo X., Yang M., Xin D., Zhang Z., Li X., Lv Ch., Liu Ch., Qi Z., Xu J., Wu X., Chen Q. 2021. Enhanced production of seed oil with improved fatty acid composition by overexpressing NAD+-dependent glycerol-3-phosphate dehydrogenase in soybean. Journal of Integrative Plant Biology 63: 1036–1053. DOI: https://doi.org/10.1111/jipb.13094

Zhou X., Ling X., Guo H., Zhu-Salzman K., Ge F., Sun Y. 2021. *Serratia symbiotica* Enhances Fatty Acid Metabolism of Pea Aphid to Promote Host Development. International Journal of Molecular Sciences 22 (11): 5951. DOI: https://doi.org/10.3390/ijms22115951