

***In Vitro* effects of cuticular lipids of the aphids *Sitobion avenae*,
Hyalopterus pruni and *Brevicoryne brassicae* on growth and
sporulation of the *Paecilomyces fumosoroseus* and *Beauveria
bassiana***

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Abstract

The exoskeleton lipids of three dangerous pests, *Sitobion avenae*, *Hyalopterus pruni* and *Brevicoryne brassicae* were identified by GC/MS studies. The main components found were triacylglycerols with one hexanoyl group. Fatty acid composition and position of triacylglycerols were determined from mass spectra. There was a trace of triacylglycerol with the E,E-2,4-hexadienoyl group in the extract of *Brevicoryne brassicae*. A series of hydrocarbons and free fatty acids were identified. Single components of straight chain aldehyde (C₃₀) and alcohol (C₃₂) were detected in the lipids of *H. pruni*, *B. brassicae*. Free fatty acids, including these found in the aphid lipids, were subjected to fungi-insect ecological studies. A homologous series of acids were added individually into media used to evaluate the mycelia growth and sporulation tests of the fungi, *Beauveria bassiana* and *Paecilomyces fumosoroseus*. The tests were performed in vitro and linear mycelial growth and sporulation of fungi after 14 days were measured. For both fungi, complete inhibition was observed with pentanoic and sorbic (E,E-2,4-hexadienoic) acids at a concentration as low as 0.02% w/v in both tests. Growth stimulation effects were only observed for *B. bassiana* with tetradecanoic and eicosanoic acid. Inhibitions were noticed for both fungi and the strongest effects were for dodecanoic (*B. b.*) and eicosanoic acid (*P. f.*). Beside free fatty acids, no other group of chemical compounds was found in the lipids which could be involved in aphids resistance to entomopathogenic fungi.

Keywords: Aphids, *Hyalopterus pruni*, *Brevicoryne brassicae*, *Sitobion avenae*, entomopathogenic fungi, sporulation, growth of, exoskeleton lipids, fatty acids

Introduction

Although chemical pesticides continue to be the main pest control agents, interest in biopesticides is growing, as they show promise in the protection of agriculture crops. Some biopesticides are already applied commercially and some are undergoing laboratory and field tests. A range of commercial biopesticides include those with bacteria, fungi, nematodes, protozoa or viruses as active ingredients. Biopesticides however, are often not broad spectrum pesticides and frequently restricted to specific species.

Aphids are major pests of temperate agriculture and horticulture, causing damage either directly by feeding on the plants or by serving as vectors for plant viruses. The aphid species chosen for this study, *Sitobion avenae* (F.), *Hyalopterus pruni* (Geoff.) and *Brevicoryne brassicae* (L.), feed on cereals, plum trees and cabbages, respectively. The entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Paecilomyces fumosoroseus* (Wize) Brown et Smith were determined earlier to be very efficacious biopesticides against thrips and whiteflies, but ineffective against some aphids.^{1,2,3} *B. bassiana* is an effective mycoinsecticide which virulence capability against *Galleria mellonella* and *Trichoplusia ni* have been studied.⁴

Aphids are subject to predation, parasitism or competition from other organisms, and it may be feasible to use all or some of these ecological interactions to control the population of these insects. Understanding of the interactions can lead to the identification of many potential opportunities for the use of living organisms as practical bioinsecticides. The importance of chemical ecology in tritrophic interactions between aphids and aphidiid parasitoid wasps has been demonstrated.⁵ These interactions are mediated by aphid semiochemicals.

Our paper is devoted to the study of lipids of some aphid species and their influence on the development of two entomopathogenic fungi of *B. bassiana* and *P. fumosoroseus* and to understand why the fungi are not good control agents for some aphids we decided to investigate the molecular level of aphid-fungi ecological interaction, as available information is still insufficient. The experiments were performed for homologous series of fatty acids similar to Kerwin⁶, who studied the ecology of Diptera and the fungus *Entomophthora culicis*. He found that the cuticular lipid components can selectively stimulate or inhibit the germination even from the same homologous series.

Methods and materials

Juveniles and adults of aphids: *S. avenae*, *H. pruni* and *B. brassicae* were collected from host plants. An extract of lipids was obtained by immersing 700 aphids of each species in 20 ml of n-hexane followed by 20 ml of chloroform/methanol mixtures (2:1 = V/V) for 5 minutes. The combined solutions of hexane and chloroform extracts were the subjects of analytical studies after evaporation.

Derivatives

Direct silylation. Crude extract (0.5 mg) was treated with 50 μ l of a mixture of bis(trimethylsilyl)-acetamide (BSA) and 15 % chlorotrimethylsilane (TMCS) at 100°C for 10 min.

Alkalic hydrolysis followed by silylation. Crude extract (0.7 mg) was dissolved in 100 μ l of 0.5 N KOH in methanol and heated for 3h at 70°C. Afterwards, the samples were dried in a nitrogen stream and treated with silylation reagents as mentioned.

Gas chromatography (GC) analysis

GC analyses were carried using a Varian Aerograph 1400 gas chromatograph modified to accept a capillary column. A 30 m DB-1 capillary column with an internal diameter of 0.28 mm was used. For total profiles of the extracts, a glass capillary column (12m, 0.3mm ID) was used. Argon was used as the carrier gas. The Kovats retention indexes were determined at 250 or 270°C. The retention index measurements error was ± 1 . The k' values for retention index estimation were more than 5.

Mass spectrometry

GC/MS measurements were carried out with a VG Micromass 7070E mass spectrometer coupled with a Dani 3800 gas chromatograph or VG Micromass 16F spectrometer linked to a Pye Unicam gas chromatograph. The mass spectra were recorded at 70 eV ionization energy.

Hydrocarbons were identified by mass spectra and Kovats retention indices, and by coinjection with standards. The positions of double bonds in the chains of unsaturated fatty acids were determined by coinjection of the lipids with the proper standard compounds (*Z*-9-octadecenoic and *Z,Z*-9,12-octadecadienoic acid - Sigma Chemical Co.).

Fungi and culture conditions

The strain of the fungi used in these studies *Beauveria bassiana* (PIK-3) and *Paecilomyces fumosoroseus* (PIK-2) (authors collection) were obtained from infected larvae of *Stilpnotia salicis* L. (Lepidoptera: Lymantriidae). These were collected at Kunowo (near Poznań), Poland. Growth studies were performed on Sabouraud medium containing 0.02% of a test acid. The acids used in the tests were: pentanoic, ethane-1,3-dicarboxylic, hexanoic, 4-methylpentanoic, *E,E*-2,4-hexadienoic, dodecanoic, tetradecanoic, hexadecanoic, heptadecanoic, octadecanoic, *Z*-9-octadecenoic, *Z,Z*-9,12-octadecadienoic, *Z,Z,Z*-9,12,15-octadecatrienoic, and eicosanoic acid, one acid incorporated in one medium test. Each, growth or sporulation, test was repeated five times. The fungi were grown on Petri dishes (9 cm diameter) which were filled with prepared media. The media containing thermostable acids were autoclaved for 20 min at 121°C. The thermounstable solutions of unsaturated acids were sterilized by filtration through Millipore filters and added to autoclaved medium. The medium without acid was used as control. The fungal spores were inoculated on the medium at 6.5 million per dish. Afterwards, the dishes were kept for 14 days at 23°C and linear growth of mycelium at 2, 5, 9 and 14 days was determined. Sporulation efficiency was estimated on day 14 and expressed in millions of conidia per 1 cm² of the colony. The mycelia were homogenised, diluted with 100 cm³ and spores were counted using a hemocytometer. The concentration of spores in 1 cm³ was calculated according to where:

$$T = \frac{\sum n \cdot P}{m} \cdot 250000$$

Σn is a sum of spores in all squares,

P - dilution factor,

m - number of squares.

The resulted figures were converted into concentrations in 100 cm³ of water.

Two tests, linear colony mycelial growth and sporulation, were used in this study. Studies were performed on a Sabouraud medium which contained peptone and glucose as sources of nitrogen and carbon, respectively, in all experiments. The experiments were designed to explain what effect lipid components may have on fungal growth at the concentration as low as 0.20%. For the primary study we chose fatty acids, as their effects had been demonstrated for 1% solutions.⁷

Results and Discussion

The extraction of the aphids studied provided small samples, ca. 2 mg per 700 insects. This restricted the choice of analytical techniques which could be used in the study.

Direct GC/MS analyses provided the chemical identity of GC peaks. Two major groups of chemical compounds, triacylglycerols (TAG), alkanes and fatty acids were found. Besides, one aldehyde (C₃₀) and one alcohol (C₃₂) were identified in *H. pruni* and *B. brassicae* lipids.

The mass spectra of the major peaks were characteristic of TAG structures. The following ions were found (Table 1) in the spectra:

- (i) RCO⁺ [F]⁺ ions of fatty acids present in TAGs,
- (ii) fragment ions of fatty acids with glycerol moieties ([F+74]⁺; [F+115]⁺ and [F+128]⁺),
- (iii) ions containing glycerol linked with two complete fatty acid moieties [M-OAcyl]⁺ and
- (iv) ions [M-CH₂OAcyl]⁺.

The identification of the ions enabled us to assign the structures of individual TAGs (Table 1). In all lipids studied, the main compound is triacylglycerol with one C₆ and two C₁₄ moieties. The position of acyl groups can be deduced from the [M-CH₂OAcyl]⁺ ions. Longer fatty acids are linked at C₁ and C₃ carbons of glycerol, but C₆ acid in the middle, as the ions [M-CH₂OAcyl]⁺ were only identified for longer fatty acids.⁸

Table 1. Triacylglycerols of aphid's exoskeleton lipids and their partial mass spectra characteristic ions m/z

Aphid	Fatty acids composition	Percent composition	[RCO] ⁺	[RCO +74] ⁺	[RCO +115] ⁺	[RCO +128] ⁺	[M-RCOO] ⁺	
<i>Sitobion</i>	C ₁₂ C ₆	9	99, 183,	173,	214, 298, 326	227,	355,	
	C ₁₄		211	257,		311,	383, 467	
<i>avenae</i>	C ₁₄ C ₆	86	99, 211	173,	214, 326	227,	383, 495	
	C ₁₄			285		339		
	C ₁₄ C ₆	5	99, 211,	173,	214, 326,	227,	383,	
C ₁₆	239		285,	354		339,		411, 495
<i>Hyalopterus</i>	C ₁₂ C ₆	8	99, 183,	173,	214, 298,	227,	355,	
	C ₁₄		211	257,		326	311,	383, 467
<i>pruni</i>	C ₁₄ C ₆	54	99, 211	173,	214, 326	227,	383, 495	
	C ₁₄			285		339		
	C ₁₄ C ₆	32	99, 211,	173,	214, 326,	227,	383,	
C ₁₆	239		285,	354		339,		411, 523
<i>Brevicoryne</i>	C ₁₆ C ₆	6	99, 239	173,	214, 354	227,	411, 551	
	C ₁₆			313		367		
	C ₁₂ C ₆	1	99, 183	173,	214,	227,	355, 439	
C ₁₂ C ₁₀	99, 155,		257	298, 214,		311		327,
C ₆ C ₁₄	211		173,	270, 326		227,		383, 439
<i>brassicae</i>	C ₁₂ C ₆	9	99, 183,	173,	214, 298,	227,	355,	
	C ₁₄		211	257,		326	311,	383, 467
				285		339		
	C ₁₄ C _{6:2}	trace	95, 211	285	326	339	379, 495	
	C ₁₄							
	C ₁₄ C ₆		56	99, 211		173,	214, 326	227,
C ₁₄		285		339				
C ₁₄ C ₆	28	99, 211,	173,	214, 326,	227,	383,		
C ₁₆		239	285,		354		339,	411, 523
C ₁₆ C ₆	6	99, 239	173,	214, 354	227,	411, 551		
C ₁₆			313		367			

Table 2. Cuticular hydrocarbons of the aphids *Brevicoryne brassicae* (*B.b.*) and *Hyalopterus pruni* (*H.p.*)

No.	Hydrocarbon	KI*	% of total cuticular hydrocarbons		Characteristic ions m/z
			<i>B.b.</i>	<i>H.p.</i>	
1	n-heneicosane	2100		5.9	296 [M] ⁺
2	n-docosane	2200		1.6	310 [M] ⁺
3	n-tricosane	2300		1.0	324 [M] ⁺
4	3-methyltricosane	2374		6.7	309 [M-29] ⁺
5	n-pentacosane	2500	2.5	36.5	352 [M] ⁺
6	2-methylpentacosane	2564		2.1	351 [M-15] ⁺ , 323 [M-43] ⁺
7	3-methylpentacosane	2574		15.7	337 [M-29] ⁺
8	n-hexacosane	2600	1.5	1.5	366 [M] ⁺
9	2-methylhexacosane	2664	11.6		365 [M-15] ⁺ , 337 [M-43] ⁺
10	n-heptacosane	2700	31.9	7.5	380 [M] ⁺
11	7-methylheptacosane	2746	12.1		112/113, 309 [M-85] ⁺
12	3-methylheptacosane	2772	9.3	2.6	365 [M-29] ⁺
13	n-nonacosane	2900	23.8	7.0	408 [M] ⁺
14	11-methylnonacosane	2938		5.2	168/169, 280/281
15	7-methylnonacosane	2944	5.1		112/113, 337 [M-85] ⁺
16	2-methyltriacontane	3062		4.0	421 [M-15] ⁺ , 393 [M-43] ⁺
17	n-hentriacontane	3100	2.2	2.6	436 [M] ⁺
	Σ Hydrocarbons as %	Σ TAG =	6	15	

*Kovats' retention index

$$KI = 100z + 100 \left(\frac{\log t_s - \log t_z}{\log t_{z+1} - \log t_z} \right)$$

where: t_s , t_z and t_{z+1} - netto retention time of the compound of interest, and standards with z and $(z+1)$ carbon numbers of n-alkanes.

Table 3. The composition of free fatty acids of aphids' cuticular lipids

No	Silyl derivative of acid	% of total free fatty acids			Characteristic ions m/z
		<i>B. brassica</i>	<i>H. pruni</i>	<i>S. avenae</i>	
1	hexanoic*	16.5	3.3		
2	dodecanoic			4.5	272 [M] ⁺ , 257 [M-15] ⁺
3	tetradecanoic	21.0	30.0	72.0	300 [M] ⁺ , 285 [M-15] ⁺
4	hexadecanoic	39.0	30.0	2.5	328 [M] ⁺ , 313 [M-15] ⁺
5	<i>Z,Z</i> -9,12-octadecadienoic	7.0	13.7	12.0	352 [M] ⁺ , 337 [M-15] ⁺
6	<i>Z</i> -9-octadecenoic			9.0	354 [M] ⁺ , 339 [M-15] ⁺
7	octadecanoic	16.5	21.0		356 [M] ⁺ , 341 [M-15] ⁺
	Σ FFA as % of Σ TAG	15	4.5	18	

*detected only for underivatized sample

Table 4. Effects of fatty acids on linear colony growth of *B. bassiana* expressed as the ratios to control growth on days 2, 5, 9 and 14

No	Day 2		Day 5			Day 9			Day 14		
	Mean	<i>Wp</i>	No	Mean	<i>Wp</i>	No	Mean	<i>Wp</i>	No	Mean	<i>Wp</i>
4	0.000		4	0.000		4	0.000		4	0.718	
6	0.000	0.217	3	0.628	0.114	3	0.758	0.084	3	0.820	0.089
12	0.000	0.228	12	0.907	0.120	1	0.804	0.088	7	0.936	0.093
3	0.194	0.233	7	0.919	0.122	2	0.812	0.090	1	0.936	0.095
7	0.903	0.240	5	0.977	0.126	10	0.828	0.093	2	0.962	0.098
13	1.000	0.245	1	0.977	0.128	8	0.875	0.094	8	0.962	0.100
1	1.032	0.248	11	1.000	0.130	7	0.906	0.096	11	0.994	0.101
5	1.129	0.251	13	1.000	0.132	11	0.914	0.097	10	1.000	0.103
11	1.161	0.253	6	1.047	0.133	9	0.992	0.098	13	1.000	0.104
2	1.516	0.254	2	1.070	0.134	12	1.000	0.098	6	1.077	0.104
10	1.677	0.256	10	1.081	0.134	13	1.000	0.099	9	1.103	0.105
8	1.774	0.257	8	1.128	0.135	5	1.047	0.099	12	1.128	0.105
9	1.968	0.259	9	1.198	0.136	6	1.078	0.100	5	1.160	0.106

The entries (No) correspond to acids:

- ethane-1,2-dicarboxylic, 2. 4-methylpentanoic, 3. hexanoic, 4. dodecanoic, 5. tetradecanoic, 6. hexadecanoic, 7. heptadecanoic, 8. *Z,Z,Z*-9,12,15-octadecatrienoic, 9. *Z,Z*-9,12-octadecadienoic, 10. *Z*-9-octadecenoic, 11. octadecanoic, 12. eicosanoic, 13. control (without an acid).

The data of pentanoic and E,E-2,4-hexadienoic acids (full inhibition of growth) were deleted from the Duncan's multiple range test analysis.

$$W = r(\alpha_p, p, k(n-1)) \sqrt{MSE / n}$$

where - $r(\alpha_p, p, k(n-1))$ are tabulated (Puri et Mullen, 1980),

k - are the numbers of acids used,

n - the number of observations per sample,

α_p - protection level (0.05),

MSE - error mean square.

Table 4a. Effects of fatty acids on linear colony growth of *P. fumosoroseus* expressed as the ratios to control growth on days 2, 5, 9 and 14

Day 2			Day 5			Day 9			Day 14		
No	Mean	W_p	No	Mean	W_p	No	Mean	W_p	No	Mean	W_p
4	0.000		4	0.000		4	0.621		12	0.592	
3	0.119	0.210	3	0.654	0.117	12	0.681	0.074	4	0.728	0.073
1	0.576	0.221	1	0.790	0.123	1	0.732	0.078	1	0.755	0.077
12	0.678	0.226	2	0.821	0.126	11	0.740	0.080	11	0.762	0.079
6	0.745	0.233	12	0.864	0.130	6	0.800	0.082	6	0.768	0.081
2	0.763	0.237	6	0.876	0.132	10	0.843	0.084	8	0.796	0.083
7	0.780	0.240	11	0.889	0.134	5	0.860	0.085	10	0.870	0.084
5	0.813	0.243	7	0.926	0.135	2	0.885	0.086	2	0.925	0.085
13	1.000	0.245	5	0.926	0.137	8	0.894	0.087	5	0.925	0.086
8	1.051	0.246	8	0.951	0.138	3	0.911	0.087	7	0.938	0.086
11	1.084	0.248	10	0.982	0.138	7	0.928	0.088	9	1.000	0.087
10	1.186	0.249	13	1.000	0.139	13	1.000	0.088	13	1.000	0.087
9	1.390	0.251	9	1.012	0.140	9	1.072	0.089	3	1.020	0.088

The entries (No) correspond to acids as was mentioned under Table 4.

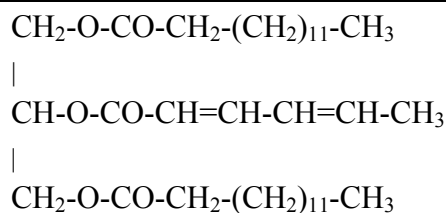
Table 5. Fatty acid influence on sporulation capacity of *B. bassiana* (*B.b.*) and *P. fumosoroseus* (*P.f.*) on day 14

<i>B.b.</i>			<i>P.f.</i>		
No	Mean	<i>Wp</i>	No	Mean	<i>Wp</i>
4	0.430		3	0.422	
2	0.674	0.392	7	0.471	0.392
8	0.755	0.413	9	0.481	0.413
3	0.784	0.422	4	0.489	0.422
10	0.885	0.435	8	0.490	0.435
13	1.000	0.442	6	0.595	0.443
11	1.109	0.448	11	0.719	0.449
7	1.114	0.453	2	0.728	0.454
9	1.155	0.457	5	0.765	0.458
12	1.159	0.460	10	0.784	0.460
1	1.330	0.463	1	0.894	0.463
6	1.344	0.466	13	1.000	0.466
5	1.493	0.468	12	1.687	0.469

The entries (No) correspond to acids as was mentioned under Table 4

In two species, *H. pruni* and *B. brassicae*, a second abundant nonsymmetrical triacylglycerol (TAG) was found with C₆, C₁₄ and C₁₆ fatty acids. This TAG is missing in the extract of *S. avenae*. But the major triacylglycerols in all lipids studied are those with tetradecanoic acid. The numbers of TAGs isomers with different fatty acid compositions present in triacylglycerols (Table 1) were 3, 4 and 7 in the extracts of *S. avenae*, *H. pruni* and *B. brassicae* respectively. The isomers were concluded from the sets of fragmentation ions (Table 1).

Beside TAGs with saturated fatty acids, a trace GC peak with a base ion at m/z 95 in the mass spectrum was found in the extract of *B. brassicae*. It can be assigned to the acyl ion of E,E-2,4-hexadienoic acid. This unsaturated TAG was found in 0.3% of ΣTAG. Thus, we conclude that this minor triacylglycerol has the structure previously described by Bowie and Cameron⁹ and Stransky et al.¹⁰:



The second most abundant group of compounds are hydrocarbons. Those of *S. avenae* have already been studied.¹¹ In both *H. pruni* and *B. brassicae*, hydrocarbons constitute a small

percentage (15% in *H.p.* and 6% in *B.b*) of the total TAG. Hydrocarbons of *S. avenae* constitute 4% of the extracts.¹¹

The compositions of hydrocarbon mixtures are presented in Table 2. n-Alkanes and branched monomethyl alkanes which form the homologous series were found. n-Alkanes n-C₂₅ and n-C₂₇ are the major components in *H. pruni* and *B. brassicae* hydrocarbon fractions, respectively. In isoalkane series, the methyl branching points are at C₂, C₃, C₇ or C₁₁ carbon atoms. The structures of branched hydrocarbons deduced from mass spectra and Kovats' retention indices are completely correlated with the known results of their biosyntheses. The hydrocarbon with an even carbon number branched at C₂, 2-methylhexacosane, has a total number of 27 (odd) carbon atoms while 7-methylheptacosane has 28 (even).¹²

Free fatty acids were detected as underivatized compounds and as trimethylsilyl esters by GC and GC/MS, and their compositions are shown in Table 3. Free fatty acids of *S. avenae* differ from the other two species. Nevertheless, the homologous series of normal saturated and unsaturated, monocarboxylic acids extending from C₁₂ to C₁₈ with the maxima at C₁₄ or C₁₆ were found in all three species. Hexanoic acid was detected in underivatized samples. No trace of E,E-2,4-hexadienoic acid was found in any of the extracts studied.

Free alcohols of the aphid extracts were studied as native compounds and as trimethylsilyl ethers. Structural assignment of free long-chain alcohol was based on M-18 and M-46 (more abundant) ions.¹³ Mass spectra of silyl derivatives showed the abundant ions (M-15)⁺, and m/z 75 and 103 revealing primary alcohols.¹⁴ Saturated C₃₂ alcohol was found in concentration 0.8%, 6.0% and 0.3% of ΣTAG in the extracts of *B. brassica*, *H. pruni* and *S. avenae*, respectively.

Long-chain aldehyde was characterized by M⁺ (weaker), M-18 (stronger) and M-46 (weaker) ions.¹⁵ An aldehyde C₃₀ was found in the extracts of *B. brassica* and *H. pruni* in the concentration of 1% and 7% of ΣTAG, respectively. Only a trace of this aldehyde was found in the *S. avenae* extract.

The fatty acids used for ecological fungi-insect studies represent saturated and unsaturated homologous series of monocarboxylic acids and one dicarboxylic acid, viz. ethane-1,2-dicarboxylic acid (succinic acid). Half of them were found in aphids lipids. Direct comparisons between fungi are difficult since control colonies grow differently for these two species. Hence proportional growths relative to control groups were calculated (Table 4 and 4a). Duncan's multiple range test was used to evaluate the effects of the acids.¹⁶ The values in rectangles are not significantly different from controls. Above and below marked figures are those which correspond to inhibition or stimulation effects, respectively. The acids were individually incorporated into media used to evaluate the mycelial growth and sporulation tests of the fungi, *B. bassiana* and *P. fumosoroseus*. The inhibition effects was total or for the initial period of the study only (Table 4 and 4a). Two acids, pentanoic and E,E-2,4-hexadienoic, completely inhibited the germination and growth for 14 days for both, *B. bassiana* and *P. fumosoroseus* fungi at a concentration as low as 0.02% w/v. Growth stimulation effects for day 14 were only observed for *B. bassiana* with tetradecanoic and eicosanoic acid. Inhibitions were noticed for

both fungi and the strongest effects were for dodecanoic (*B. b.*) and eicosanoic acid (*P. f.*). More acids inhibit growth of *P. fumosoroseus* than *B. bassiana*. Sporulation capacity (Table 5) of *P. fumosoroseus* is again more dependent (inhibition) on the acids than that of *B. bassiana*. The inhibition of sporulation of *P. fumosoroseus* is strongest by hexanoic acid. The abundant acid in aphid lipids, tetradecanoic acid, significantly stimulate the sporulation capacity of both fungi.

Discussion

The purpose of this paper was to explain the poor activity of bioinsecticides with fungi *B. bassiana* and *P. fumosoroseus* against *S. avenae*, *H. pruni* and *B. brassicae*. For this, the composition of the aphid lipids was studied. Solvent extractable lipids of the insects are very complicated mixtures of organic compounds in which the following groups of lipids can be distinguished^{17,18}: fatty acids, alcohols, esters, triacylglycerols, sterols, aldehydes, ketones and hydrocarbons. The aphid lipids were extracted from the insects with n-hexane followed by chloroform/methanol mixture. The question arises here, as to where the TAGs are from. Short extraction time suggests that TAGs are mostly from cuticular lipids, but it cannot be excluded that the fractions are partially from fat bodies and wax cells near the siphunculus bases. Triacylglycerols are not usually the major components of the cuticular lipids of most insects^{19,17}, but are the dominating class of compounds of internal lipids.²⁰ The detection of triacylglycerols in the extracts is therefore a useful indicator of the most favourable extraction procedure for complete removal of the cuticular lipids. For our study it was extremely important that all cuticular components were extracted and identified even at the cost of the sample being partly contaminated with TAGs. Pentane extraction followed by a chloroform/methanol mixture of the pea aphid¹⁰ provided mostly triacylglycerols similarly to our results. But it was later demonstrated by scanning electron microscope²¹ that 4 hr extraction with pentane left the cuticle disrupted and the epidermal cells were completely lysed. The basement membrane remained intact, but the solvent reached the hemocoel. A 5-min extraction in chloroform/methanol showed even more dramatic disruption - the epidermal cells were lysed. Moderate extraction with pentane, however provided also triacylglycerols, leaving the question of origin still not completely solved for aphid lipids.¹⁰

Triacylglycerols found in our studies are possibly of extracuticular origin. When aphids are disturbed, as they were during extraction, they secrete drops of waxy material. Analyses of this substance by Strong²² and Callow et al.²³ found it to be triacylglycerols rich in mirystic acid (C_{14:0}).

Free fatty acids even-numbered are present in all extracts studied here with distribution typical for insects, between C₁₂ (saturated) and C₁₈ (saturated and unsaturated).¹⁸ Even longer free fatty acids were reported in the insects lipids up to C₃₆.²⁴ But such short acid as hexanoic was identified there for the first time in this paper. Obviously, it is related to the fact that all TAG's contain the hexanoyl group (Table 1).

S. avenae extract contains dodecanoic acid, which was found to be a deterrent for aphid settling on the leaves. Other fatty acids show similar but weaker activity and they work slowly.²⁵ Nevertheless, the application of dodecanoic acid has been successful in reducing the level of *A. fabae* colonisation on sugar beet in field conditions.²⁶

Long chain aldehyde triacontanal and n-alcohol dotriacontan-1-ol found in *H. pruni* and *B. brassicae* differ in the chain length by two methylene groups. Similar compounds have been identified in several insect species.^{10,27,28} However, long chain aldehydes are not common constituents of insect lipids. They usually accompany long chain alcohols as was found here. The function of long chain aldehydes and alcohols in insects is not known as yet.²⁸

The insect integument is a barrier for germination of pathogens including fungi. Shimizu²⁹ demonstrated that E,E-2,4-hexadienoic (sorbic) acid, free or bonded, is present in some aphid species and is a potent fungicidal agent. However, the resistance of aphids against *B. bassiana* and *P. fumosoroseus* is not clear. Two species of aphids, *S. avenae* and *H. pruni*, do not even have a trace of E,E-2,4-hexadienoic acid. No other organic groups except free fatty acids which could be involved in insect-fungi ecology, were found.

According to Smith and Grula⁷, short fatty acids such as pentanoic and hexanoic acid inhibit germination of *B. bassiana* conidia. The cuticular components of *Heliothis zea* inhibit *Aspergillus niger* but *B. bassiana* could grow undisturbed on it.³⁰ Furthermore, the molecular background of insect-fungi ecology can be complicated by the presence of saprophytic microorganisms on the integument. The saprophytic microflora associated with the insect cuticle can either stimulate or inhibit the germination of the spore *in vivo*.³¹

As was found here, more acids inhibit the growth of *P. fumosoroseus* than *B. bassiana*. But the final sizes of mycelia of *B. bassiana* were comparable with those of controls. For day 14 and tetradecanoic and eicosanoic acid media, the sizes of mycelia of *B. bassiana* were greater than the control ones. Growths greater than controls were not observed for *P. fumosoroseus*. There were differences in sizes, the smallest growth was observed for ethane-1,2-dicarboxylic, dodecanoic, hexadecanoic and particularly eicosanoic acid.

Literature data on lipid components of aphids show a high proportion of triacylglycerols with E,E-2,4-hexadienoic acid, even as high as 50 % of total TAG.¹⁰ This may explain the low efficiency of fungal pathogens as biopesticides in some cases. However, the species of insects studied in this paper do not show even traces (except *B. brassicae*) of E,E-2,4-hexadienoic acid.

Nutritional requirements for germination and growth of *B. bassiana* were estimated.⁷ According to these authors, all short fatty acids up to nonanoic acid stopped germination, but all fatty acids including Z-9-octadecenoic inhibited growth at an acid concentration of 1%. When the concentration was lower, 0.02% as we also used, germination was inhibited only by short chain fatty acids. The concentration used in our study was lower than that found in insect lipid extracts. Herein, the conclusion can be drawn that fatty acid concentration in aphid cuticle is sufficient to stop the germination and growth of fungi. More importantly, fatty acids can show synergetic activity, which was not studied in our experiments and is not reported in the literature.

As was mentioned before, *B. bassiana* and *P. fumosoroseus* are good biopesticides against

whiteflies.¹ A study of whiteflies cuticular lipids did not reveal a trace of fatty acids there.²⁷ This is an additional evidence of fatty acids involvement in insect-fungi ecology.

The experiments presented above covered germination and vegetative growth periods, but the effect of a bioinsecticide is also dependent on sporulation efficiency. Obviously, full growth inhibition by pentanoic and E,E-2,4-hexadienoic acid is accompanied with full inhibition of sporulation. For *B. bassiana* (Table 5), the highest sporulation was observed on a medium with tetradecanoic acid but the lowest for dodecanoic acid. However for *P. fumosoroseus* the highest production was for eicosanoic acid and significantly lower for hexanoic, heptadecanoic, Z,Z-9,12-octadecadienoic, dodecanoic and Z,Z,Z-9,12,15-octadecatrienoic acid. Generally, *B. bassiana* sporulation was correlated with greater growth of mycelium, but for *P. fumosoroseus* this relation was reversed.

In our search for the molecular level of the ecological interaction between aphids and entomopathogenic fungi we did not find a compound with spectacular antifungi activity, except for a trace of TAG with sorbic acid in *B. brassicae* lipids. As previously mentioned, both fungi are not effective bioinsecticides against aphids. This can be due to the presence of free fatty acids found in their lipids. No other group of compounds could be involved in insect-fungi interaction as according to Nelson et al.²⁸ and Buckner et al.²⁷ a lot of aldehydes (C₃₂ and C₃₂) and alcohols (C₃₂ and C₃₄) were found in lipids of whiteflies. Nevertheless *P. fumosoroseus* and *B. bassiana* are good biopesticides against whiteflies.^{1,3}

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