Fatty Acid Distributions as Related to Adult Age, Sex and Diet in the Phytophagous Heteropteran, *Lygus hesperus* (Heteroptera: Miridae)[1, 2]

Allen C. Cohen

Honey Bee and Biological Control of Insects, USDA-ARS
2000 East Allen Road, Tucson, AZ 85719

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ABSTRACT The phytophagous heteropteran, *Lygus hesperus* Knight was analyzed to determine the distribution of fatty acids among lipid classes in laboratory-reared adults (newly eclosed and 5 day-post eclosion) and in field-collected adults. Profiles of lipid classes varied with source of the insect (field vs. diet-reared), age and sex. Total lipids (% dry wt.) ranged from 5.3 to 30.3% in field-collected males and 5 day-post eclosion diet reared females, respectively. Except for field males the predominant lipid class was triacylglycerols (up to 90% of the total lipids) with phospholipids generally the second most abundant lipid class. The most abundant fatty acid in all classes and all treatments was oleic acid (C18:1 ω 9). There were significant differences between the field collected vs. diet-reared insects with respect to concentrations of linolenic acid (C18:3 ω 3), with this polyunsaturated fatty acid being much more abundant in field-collected insects than in diet-reared ones.

KEY WORDS *Lygus hesperus*, lipid classes, diet.

Most insects require a dietary source of polyunsaturated fatty acids, (Dadd, 1983). However, there is a great deal of variation regarding the extent that carcass profiles correspond with dietary profiles of fatty acids (Thompson and Barlow, 1983). The degree of conformity of fatty acid profiles to diet rather than to genetically determined profiles is a measure of an organism's metabolic and dietary independence. A loss of dietary independence is probably closely correlated with adaptation to highly specialized diets (as in parasitic insects). Fatty acid profiles are also known to vary with lipid class, tissues from which they are taken or other biological factors such as age or sex of the insect (Thompson and Barlow, 1983; Stanley-Samuelson and Dadd, 1983; Stanley-Samuelson and Pipa, 1984; Stanley-Samuelson and Lohrer, 1986; Stanley-Samuelson et al., 1988; Bridges and Watts, 1975; Miller and Blankenship, 1973).

The subject of this study, *Lygus hesperus* Knight (Heteroptera: Miridae) is an economically important pest of various crops around the world. Its wide array of host plants and facultative entomophagy make it an excellent subject of nutrition/metabolism studies where adaptations to unspecialized feeding are to be investigated. Furthermore, knowledge of *L. hesperus*' biochemical profile is needed in efforts to

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grasp the nutritional relationship of this insect to its predators and parasites (i.e. what nutrients are available to its entomophages).

The major object of this study was to develop a procedure that would allow objective comparison of artificial diet-fed (AD-fed) *L. hesperus* with their wild counterparts. Secondly, this study was intended to provide needed information on the distribution and metabolism of fatty acids among the various lipid classes and in different ages and sexes of this insect. The objective of using field-collected insects was to get a baseline on lipid distribution in what are assumed to be healthy, wild *L. hesperus*. It was impossible to determine age or feeding history in wild specimens.

MATERIALS AND METHODS

The *L. hesperus* used in this study were from laboratory cultures originating in the Avra Valley ca. 25 km NW of Tucson, AZ. The colony has been maintained on artificial diet and held at 27°C and ca. 40% RH for at least 25 generations as described by Debolt (1982) and Patana (1985). The field subjects (of unknown age) were freshly collected from the same location described above in alfalfa fields. No efforts were made to compare lipid profiles from populations collected from several crops because of this species' inclination to move from one crop system to another.

Analyses were performed on whole body chloroform/methanol extracts (Bligh and Dyer, 1959) of 6 replications of 5 individuals (killed by freezing) per treatment and ground in a glass tissue grinder. Fully hydrated, freshly killed insects were used for study with corrections made for % dry weight based on previously determined but comparable samples. The chloroform layer was aspirated and transferred to a clean evaporation tube where the volume was reduced. This extract was transferred to Vac-Elute® tubes where lipids were purified by solid phase extraction with a $CHCl_3$ wash (Christie, 1982). Extracts were immediately treated with butylhydroxytoluene (BHT) (Christie, 1982) to reduce autoxidation and were used either for lipid class separation by thin-layer chromatography (TLC) or for direct transesterification described by Christie (1972) and modified by Cohen and Debolt (1984). Major lipid classes were separated on 250 μ Kieselgel G plates with a solvent system of hexane-diethyl ether-formic acid (80:20:2 v/v/v) with about 0.5 - 3 mg (based on gravimetric determination of similarly treated samples) of sample or standard used for each spot. Two techniques were used to determine that visualization techniques were not destructive to lipids of interest. After development, standard plates were co-chromatographed with separate sample containing plates. The former were visualized either with 2,4-dichlorofluorescein in ethanol or by exposure to iodine vapor. Spots or non-visualized corresponding regions from sample plates with Rf's corresponding to those of authentic standards were scraped onto solid phase extraction columns and eluted with chloroform or chloroform and methanol (for phospholipids). Recoveries from this method were found identical to those from separation of standards and samples separated on the same plate and visualized as described above. Eluates were dried under N_2 and transesterified with BF_3-methanol as described by Metcalfe et al. (1966). Methyl esters of the fatty acids from the total lipids or from individual lipid classes were dissolved in hexane and analyzed by GC on a 2 meter column packed with either 3% SE/30 or 10% DEGS on 80-100 mesh Gas-Chrom Q. Retention

times and peak areas were determined by comparison with authentic standards. Methyl esters of the fatty acids (FA) were formed with BF_3-methanol (Metcalfe et al., 1966). A known amount of internal standard (Cl5:0) was added prior to derivatization of standards and samples. Peak areas were measured with a Shimadzu CR1A® recording integrator. Recovery values and constants were determined and used as correction factors as described by Christie et al. (1970) from known quantities of authentic standards of each lipid class mentioned below both for TLC analyses and for total extracted lipids. Lipid classes studied here include phospholipids (PL) monoacylglycerols (MG), diacylglycerols (DG), triacylglycerols (TG), free fatty acids (FFA), and steryl esters (SE). These abbreviations will be used in the remaining text. It must be specified that "total lipids" as used here refers to lipids that were derivatized and analyzed without TLC separation of lipid classes. "Composite lipids" refers to the total of separated lipid classes.

Results

Diet-fed *L. hesperus* had higher composite lipid concentrations than did field specimens (Table 1). Sexually mature (5-day post-eclosion) adults had nearly 2 x the amount of lipid per mg body weight as did their newly-emerged counterparts. The principal lipid class was TG for all treatments except for field males where the predominant lipid class was comprised of FFA. The 5- day adults of both sexes had the highest TG concentrations with 251.7 and 242.6 μg/mg body weight of females and males respectively, which are 6 and 23 × the TG concentrations in the field derived counterparts. TG concentrations in 5-day adults are significantly higher than in newly eclosed adults which, in turn, are higher than in field adults.

Concentration of polar lipids and FFA are similar. Females from diet treatments had higher PL concentrations than all other treatments with newly eclosed females having significantly higher PL levels than older females. Field adults and 5-day females had highest FFA concentrations, and 5-day males had lowest FFA levels. Steryl esters and DG concentrations were the lowest of all lipid classes, except in 5-day males which at 15.9 μg/mg dry wt. SE. In all treatments SE showed numerically higher concentrations in males than in females. It is obvious from Table 1 and from other data presented here that there is no across-class trend that holds consistently throughout treatments.

Figure 1 shows lipid profiles of diet and *L. hesperus* of all treatments presented as % total recovered lipids within each class. The profiles of field insects, especially males, differ strongly from the diet profiles, especially with respect to TG, FFA and PL classes. Although the absolute magnitude of the SE differences is not great, the relative differences are even greater here than in the other lipid classes in Fig. 1. Fig. 1 reflects a rough correspondence between the carcass class composition and the AD upon which the *L. hesperus* fed. This figure also shows the predominance of TG in all AD-fed treatments.

Tables 2 and 3 show the concentrations of individual FA as a function of lipid class [PL + MG, DG and TG in Table 2; FA, SE and total lipids in Table 3] and treatment. Figure 1 presents data that demonstrate that the predominant lipid class is TG; and Tables 2 and 3 present data that demonstrate that the predominant fatty acid is C18:1.

In the polar lipids (PL and MG), AD-fed females had the highest level of FA except for C18:3 where both sexes of field adults had between 2 and 15 times the

Table 1. Lipid classes distributed among various treatments of *Lygus hesperus*. Values are expressed in μg of lipid/mg dry body weight found in each class separated by TLC and analyzed by GC.*

Treatment	Phospholipids + Monoacylglycerides	Diglycerides	Triglycerides	Free Fatty Acids	Steryl Esters	Composite† Lipids
Field ♀♀‡	15.2c	2.1bc	44.6c	18.7a	3.7a	84.3
Field ♂♂	15.9c	1.6c	10.6c	19.8a	4.9b	52.7
Newly Eclosed diet ♀♀	27.7a	3.9ab	135.4b	10.5b	3.4bc	180.9
Newly Eclosed Diet ♂♂	16.7c	3.7ab	128.7b	13.1b	3.7bc	165.9
5-Day Post-Eclosion Diet ♀♀	22.9b	2.2bc	251.7a	24.8a	1.3c	302.9
5-Day Post-Eclosion diet ♂♂ [4]	16.4c	5.0a	242.6a	5.5c	15.9a	285.2

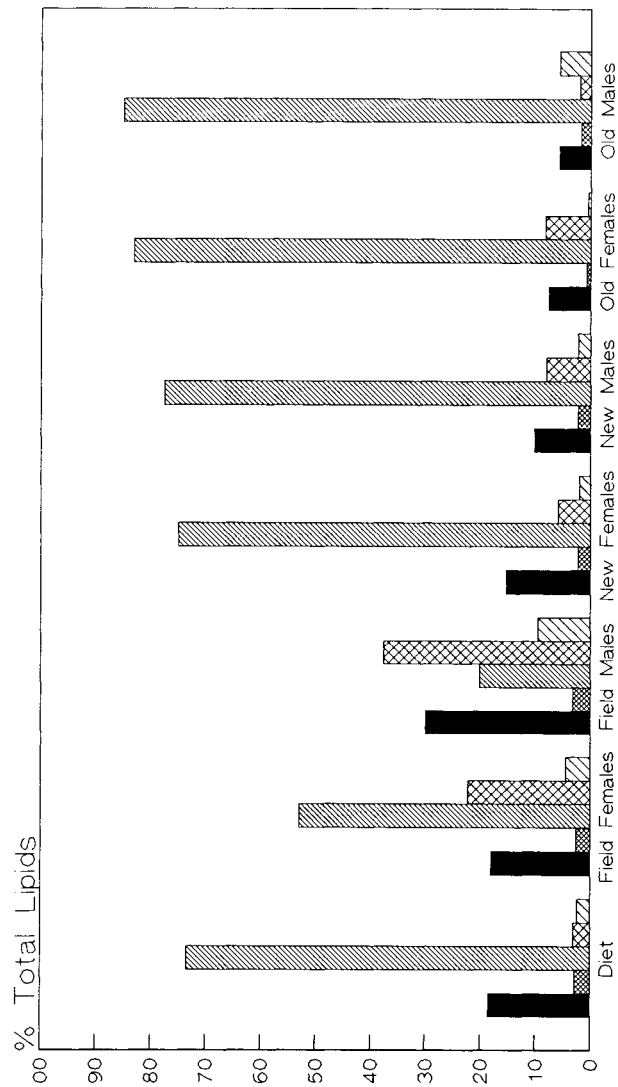

Fig. 1. A profile (in % of total lipids) of overall distribution of lipid classes in the 6 treatments of *Lygus hesperus* and the artificial diet fed to laboratory-reared subjects.

Table 2. Fatty acid composition (μg/mg dry wt.) of the phospholipid + monoacylglycerol, diacylglycerol and triacylglycerol fractions of extracts from the following treatments of *Lygus hesperus* adults: [1]=field collected females, [2]=field collected males, [3]=newly eclosed, diet-fed females, [4]=newly eclosed, diet-fed males, [5]=5-day post-eclosion females and [6]=5—day post-eclosion males.

	FATTY ACID					
(Treatments 1-6)*	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
PL & MG†						
1 [5]	0.6c	0.9a	0.8d	4.2b	2.1d	0.7a
2	0.6c	0.3b	0.9d	3.3b	3.7cd	0.8a
3	1.4a	0.9a	2.1a	6.6a	5.4a	0.3b
4	0.8c	0.5b	1.2c	4.2b	3.3c	0.2bc
5	1.2ab	0.8a	1.6b	5.9a	4.9b	0.1c
6	1.0b	0.4b	1.2c	4.2b	3.1c	0.1c
DG						
1	0.3c	0.1a	0.1b	1.0bc	0.2b	0.1a
2	0.3c	T	0.2ab	0.7c	0.3b	T
3	0.8ab	0.2a	0.3ab	2.0ab	0.5a	0.1a
4	0.7bc	0.2a	0.2ab	1.9ab	0.4a	T
5	0.5bc	0.1a	0.1b	1.1bc	0.2b	T
6	1.1a	0.2a	0.3a	2.7a	0.2b	T
TG						
1	9.7c	3.5c	4.4c	22.7c	1.3c	0.7a
2	3.0c	0.4d	1.2d	5.0c	0.4c	0.1b
3	31.1b	10.4b	8.5b	70.5b	7.5b	0.4ab
4	28.0b	8.9b	8.4b	68.6b	7.6b	0.5ab
5	54.6a	19.7a	14.3a	136.3a	13.3a	0.5ab
6	54.3a	18.0a	14.3a	129.1a	13.2a	0.8a

* Six replications per treatment unless otherwise indicated in parentheses.
† Abbreviations: PL - phospholipids; MG - monoacylglycerols; DG - diacylglycerols; TG - triacylglycerols.
‡ Trace (less than 0.1 μg/mg).

concentration of this polyunsaturated FA. Field specimens of both sexes had the lowest levels of C18:0 in their polar (PL and MG) classes. Females tended to have higher FA concentrations than did males of the same diet treatment. Except for C18:3 and C18:0 in field males, newly eclosed individuals of both sexes and 5-day males had higher FA levels than other treatments.

The most orderly pattern of FA distribution is in the predominant class, TG. Except for C18:3, 5-day post-eclosion adults had the highest concentrations, and field adults had the lowest concentrations.

The FFA class is characterized by high accumulations of FA in 5-day males (Table 1). As in the polar lipid class, the highest concentrations were found in the field adults which had levels 4.5 to 51 × as high as AD-fed adults, females in the latter group having the lowest concentrations. A clear pattern in SE FA is in the 5-day males having consistently the highest concentration of all FA types.

Total lipids were most abundant in C18:1 with relatively high concentrations of C16:0 and C18:2, the latter especially in diet-fed *L. hesperus*. Concentrations of C18:3 were highest in field specimens, especially females.

Table 3. Fatty acid composition (μg/mg dry wt.) of the free fatty acids, steryl esters, and total, unseparated lipids from [1]=field collected females, [2]=field collected males, [3]=newly eclosed, diet-fed females, [4]=newly eclosed, diet-fed males, [5]=5-day post-eclosion females and [6]=5-day post-eclosion males.

	FATTY ACID					
(Treatments 1-6)*	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
FFA†						
1	2.1b	1.6a	2.1a	6.9b	3.8ab	1.5a
2	2.5b	0.9b	2.4a	6.2cd	5.1a	1.5a
3	2.1b	0.6bc	1.0b	4.3c	1.8bc	0.1b
4	1.9b	0.9b	1.2b	5.3cd	3.0abc	0.1b
5 [4]	1.0c	0.4c	0.4c	2.4d	1.0c	T
6	4.4a	1.6a	2.3a	11.1a	3.6ab	0.3b
SE						
1 [5]	0.2b	0.1b	0.2b	0.6bc	0.3b	0.2b
2	0.2b	0.1b	0.2b	0.6b	0.6a	0.3ab
3	0.2b	0.1b	0.2b	0.5b	0.1bc	0.3ab
4	0.3b	0.1b	0.3b	0.6b	0.2bc	0.3ab
5	0.1b	T	0.1b	0.3bc	0.1c	T
6	1.3a	0.2a	1.6a	2.3a	0.7c	0.5a
TOTAL LIPIDS†						
1	22.0d	14.0d	14.1de	71.8d	23.3c	13.4a
2	13.4d	5.3e	9.5e	39.0d	29.2c	9.9b
3	66.8c	26.1bc	21.1bc	177.5b	39.3b	2.3d
4	57.7c	22.3c	17.5cd	136.6c	30.2c	2.0d
5	82.2b	31.3b	24.5b	210.7b	40.3b	1.0e
6	112.4a	43.6a	30.8a	313.2a	63.5a	4.0c

* Six replications per treatment unless otherwise indicated in parentheses.
† Abbreviations: FFA - free fatty acids; SE - steryl esters
‡ Total lipids directly extracted (not separated into lipid classes), derivatized and analyzed by GLC.
T trace (less than 0.1 μg/mg).

Discussion

Lipids play key roles in insects' biochemistry as sources of energy, structural components and as hormones (Stanley-Samuelson et al., 1988). Generally TG are major sources of energy storage while the polar lipids, especially PL, function as structural components of membranes. Lipid classes and their fatty acid moieties vary in concentration in relation to such factors as age of insects or developmental stage (Muncio, et al. 1980; Pagani et al. 1980) or according to sex or specific tissues (Stanley-Samuelson, 1984 and Stanley-Samuelson et al., 1988). There is mounting evidence that membranes performance is related to FA distribution in membrane phospholipids and their unsaturated FA moieties. In this context, and in a perspective of nutritional ecology, the distribution of FA among lipid classes and within various treatments in *L. hesperus* is of interest.

All AD-fed *L. hesperus* had higher TG contents than did field collected individuals, and as adults matured, fat content increased nearly 2-fold. The difference in composite lipid content (Table 1) in field males vs. females was not evident in either group of AD-fed *L. hesperus* (1 or 5 day). It should be noted that in this species, sexual maturation is completed about 5 days after adult eclosion. It

was unexpected that male AD-fed individuals from both age groups had composite lipid contents similar to those of comparably treated females since females accumulate material to be incorporated into maturing eggs.

This study shows that there is a considerable FA variation resulting from diet, age and source of *L. hesperus*. The most impressive and probably the most significant variation is in C18:3 acid content of field vs. AD-fed adults. It is evident from the diet FA profile (Table 4) that C18:3 was scarce in the AD and in the *L. hesperus* fed the AD.

The profiles of the diet-fed *L. hesperus* reflect the dearth of C18: 3 but not the similarly low dietary level of C16:1. The concentration of C18:3 in the diet was about 2.5% of the total fatty acids and in newly eclosed diet-fed males was about 0.7%. In contrast to this, the dietary concentration of C16:1 was about 3% while the carcass composition of Ad-fed males was about 8%. The values for 1 day females and 5 day AD-fed *L. hesperus* of both sexes were nearly identical. This could be explained as an ability of *L. hesperus* to synthesize or sequester C16:1, but not C18:3.

The difference in the ability of seed-eating vs. leaf-eating insects to absorb dietary TG (neutral lipids) vs. polar lipids found more commonly in leaf tissues has been discussed by Turunen (1983) who concluded that the FA requirements of certain insects can be met more readily if the FA in question were moieties of polar lipids such as phospoacylglycerols. In the present study, it is clear that C18: 3 was sparse especially in the polar lipid class within the AD and the AD-fed *L. hesperus*. The fact that this dearth was not found in field-collected specimens may indicate a requirement for this FA and a borderline deficiency in AD-fed *L. hesperus*. Finally, it is intriguing that the results of this study may be a first account of insects evidencing excess body fat accumulation. It is not evident, however, whether the AD-fed subjects were opportunistically storing fat as an adaptation towards periods of food scarcity or if the fat accumulation represents a deviation in lipid metabolism resulting from dietary imbalance. Further studies on feeding choices and quantities should be undertaken to resolve this issue.

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Table 4. Fatty acid composition (μg/mg dry wt.) of *Lygus hesperus* diet (Debolt) as individual lipids and total lipids (x ± SD) N = 10 samples of each treatment.

Fatty Acid	Phospholipids and Monoglycerides	Diglycerides	Triglycerides	Free Fatty Acids	Steryl Esters	Total Lipids
C16:0	4.7 (0.7)	00.7 (0.2)	15.6 (2.8)	0.8 (0.1)	0.5 (0.4)	29.6 (2.5)
C16:1	0.2 (0.1)	0	2.1 (0.4)	0	0	4.0 (0.4)
C18:0	2.1 (0.3)	0.2 (0.02)	5.0 (1.9)	0.6 (0.1)	0.5 (0.5)	9.3 (0.8)
C18:1	4.0 (0.6)	0.76 (0.1)	25.3 (6.0)	0.5 (0.04)	0.4 (0.03)	48.3 (3.7)
C18:2	2.0 (0.3)	0.41 (0.1)	3.8 (1.5)	0.3 (0.04)	0.1 (0.02)	23.1 (2.1)
C18:3	0.1 (0.1)	0	0.4 (0.1)	0	0	2.9 (0.3)

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