Effects of Dietary Conjugated Linoleic Acid on European Corn Borer (Lepidoptera: Crambidae) Survival, Fatty Acid Profile, and Fecundity

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ABSTRACT Conjugated linoleic acid (CLA) is an unusual fatty acid produced by fermentative bacteria in the rumen of ruminant mammals. Positive biological effects, including anticarcinogenic, antiatherogenic, and immune enhancing effects, have been observed in mammals fed CLA-enriched diets. Little is known of the biological effects of dietary CLA on insects, and nothing is known of the dietary CLA effects on the fatty acid profile of an insect. In this study, we examined the effects of a CLA or safflower oil-enriched meridic diet at several concentrations on European corn borer, *Ostrinia nubilalis* (Hübner), survival, development, fatty acid profiles, and fecundity. The fatty acid profiles of pupal and adult tissues as well as eggs from adults fed CLA-enriched diets as larvae were studied. Control insects were fed the meridic diet with the solvent carrier added. We hypothesized a CLA-enriched diet, but not a safflower oil-enriched diet, would decrease survival, alter fatty acid profiles, and decrease fecundity. Larvae fed the CLA-enriched diet developed more slowly than did larvae fed the safflower oil-enriched diet or the control diet. Pupal mass was not affected by any of the treatments. Survival was decreased greatly in larvae fed the CLA-enriched diet. Saturated fatty acids increased proportionately, whereas polyunsaturated and monounsaturated fatty acids decreased proportionately in both pupal and adult tissues. Fecundity was not affected by any of the treatments.

KEY WORDS conjugated linoleic acid, fatty acids, *Ostrinia nubilalis*

Nontarget pest toxicity, environmental persistence, and mammalian neurotoxicity, among other factors, of the traditional pesticides have initiated a push for more environmentally friendly, biorational pest control products. Conjugated linoleic acid (CLA) is an unsaturated fatty acid of 18 carbon length produced naturally by microorganisms in the rumen of ruminant mammals as an intermediate of the biohydrogenation of primarily linolenic acid (C18:3) or linoleic acid (C18:2) to stearic acid (C18:0). CLA refers to a group of positional and geometric isomers of C18:2.

Adverse effects by dietary CLA on reproduction have been observed in several poultry species. Hens fed diets containing 5% CLA had a lower egg production rate than did hens fed a 0 or 2.5% CLA diet (Ahn et al. 1999). Complete embryo mortality was found in eggs from hens fed a 0.5% CLA diet (Aydin et al. 2001).

Fatty acid profile alterations have been demonstrated in the eggs of hens fed CLA-enriched diets. Egg yolk lipids from hens fed a CLA-enriched diet have higher concentrations of saturated fatty acids and decreased concentrations of monounsaturated and polyunsaturated fatty acids compared with those from hens fed control diets (Chamruspollert and Sell 1999, Du et al. 1999, Watkins et al. 2003). These alterations imply the inhibition of a Δ9-desaturase. Insecticides with novel modes of action are highly desirable. Few insecticides target lipid metabolism or biosynthesis. Currently, the tetronic acid insecticides, such as spirodiclofen and spiromesifen, are the only known insecticides that affect lipid biosynthesis (Bretschneider et al. 2003). Based on previous CLA research, we believe CLA has potential to be a pest control agent. CLA effects lipid metabolism in mammals and poultry, but very little is known of the effects of CLA on insects. House fly, *Musca domestica* L., larvae and adults fed a CLA-enriched diet had CLA isomers incorporated into the tissue lipids with no negative impacts on survival, development, or reproduction (Park et al. 2000). Fatty acid profiles of the flies were not reported. Diet plays a critical role in the fatty acid composition of an insect. We hypothesized dietary CLA would alter fatty acid profiles, specifically by increasing saturated fatty acids and decreasing monounsaturated and polyunsaturated fatty acids, and that these alterations would negatively impact survival, development, and fecundity of the European corn borer, *Ostrinia nubilalis* (Hübner), an economically important crop pest. Our objectives for this study were to monitor the survival and development; quantify the individual fatty acids of pupae, adults, and eggs; and to measure the adult fecundity and egg fertility of *O. nubilalis* larvae fed a CLA-enriched diet.

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Materials and Methods

Experimental Design. This study used a completely randomized design with a 2 by 5 factorial treatment design. The two levels of the first factor were CLA and safflower oil, respectively. The five levels of the second factor were the concentrations of the compounds in the meridic diet. A control also was used, giving a total of 11 treatment combinations.

Diet Preparation and Insects. Standard meridic diet of wheat germ was provided by the Corn Insects and Crop Genetics Research Unit, Ames, IA. Diet was prepared following the method of Lewis and Lynch (1969). The appropriate amounts of CLA (Loders Croklaan, Wormerveer, The Netherlands) or safflower oil (Spectrum Organic Products, Inc., Petaluma, CA) to constitute 0.6, 0.4, 0.2, 0.1, and 0.05% (calculated as wt:vol liquid diet) were dissolved in 8 ml of acetone and incorporated into 400 ml of liquid meridic diet. Acetone (8 ml) was used for the control. The diet aliquots were stirred by hand for 1 min to ensure equal distribution of the additive. The CLA product contained primarily the *cis*-9, *trans*-11 (c9, t11) and *trans*-10, *cis*-12 (t10, c12) isomers in a 50:50 ratio. Approximately 5 ml of diet from each mixture were poured into individual 18.5-ml plastic diet cups (Fill Rite, Newark, NJ). The diet cups sat for 24 h to allow the acetone to evaporate. One *O. nubilalis* neonate larva, provided by the Corn Insects and Crop Genetics Research Unit, was placed on the surface of the diet in each diet cup. A lid was placed on each diet cup, and the diet cups were kept in an environmentally controlled room at 27°C in constant light and 75% RH.

Development Parameters. Ninety *O. nubilalis* neonate larvae (one per diet cup) were used for each treatment combination, including the control, with each larva considered a replicate. The larval duration and pupal mass (milligrams) of the survivors were recorded; the uneaten food and excrement were discarded, and the pupa was returned to the cup. The duration from hatch to adult emergence was recorded. Some adults never emerged; thus, developmental time to the adult stage was not recorded.

Survival. One *O. nubilalis* neonate larva was placed in each diet cup. An experimental unit consisted of 30 diet cups, and each experimental unit was replicated three times. The percentages of survivors to the pupal and adult stages were recorded for each experimental unit. LC_{50} values were calculated on the basis of mortality recorded at both the pupal and adult stages. The slopes and intercepts of the concentration-response curves, based on the data recorded at both pupal and adult stages, were determined.

Total Lipid Extraction and Fatty Acid Analysis. The fatty acid profiles of pupae that had been fed a 0.6, 0.4, 0.2, 0.1, 0.05, or 0.0% CLA or safflower oil diet as larvae were determined. The fatty acid profiles of male and female adult moths that had been fed a 0.2, 0.1, 0.05, or 0.0% CLA or safflower oil diet as larvae also were determined. Adults fed the two higher dietary concentrations of CLA or safflower oil were not studied because of low percentage survival of adults fed the CLA diet at these concentrations. The fatty acid profiles of eggs produced by adult males and females fed a 0.2, 0.1, 0.05, or 0.0% CLA diet as a larva were determined. An experimental unit consisted of five whole pupae or adults and each experimental unit was replicated two or three times. For the determination of egg fatty acid profiles, 500 mg of egg masses comprised an experimental unit and each experimental unit was replicated three times. Whole pupal or adult bodies were weighed and transferred to a glass homogenizer containing chloroform:methanol (2:1, vol:vol). Total lipid was extracted following the procedure of Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared from the total lipid extract by adding 2 ml of 0.5 N methanolic base (Sigma-Aldrich, St. Louis, MO) to a Teflon-lined screw cap test vial containing 10 mg of the total lipid sample dissolved in 1 ml of toluene. The reaction vial was heated at 70°C for 15 min. After cooling to room temperature, 1 ml of water and 1 ml of hexane were added. The upper (organic) layer was removed and dried over anhydrous sodium sulfate. FAME were quantified by a gas chromatograph (model 3350, Varian, Palo Alto, CA) equipped with a SP-2560 fused silica capillary column (100 m by 0.25 m by 0.2-μm film thickness, Supelco, Bellefonte, PA) and a flame ionization detector. The column was started at 70°C, held for 4 min, increased 13°C per min to 175°C, held for 27 min, increased 4°C per min to 215°C, and held for 28 min. The injector and detector were maintained at 220°C. Sample FAME were identified by comparing the retention time with those of FAME standards (Nu-Chek-Prep, Inc., Elysian, MN). Fatty acid compositions were determined using the peak areas and presented as a weight percentage. The Δ9-desaturase activity was estimated by relating the percentage of the products (oleic [18:1] and palmitoleic [16:1] acids) to the percentage of the parent compounds (stearic [18:0] and palmitic (16:0) acids] (Zhang et al. 2007). The Δ9-desaturase index was calculated by multiplying 100 times the ratio of the sum of 18:1 and 16:1 to the sum of 18:1, 18:0, 16:1, and 16:0.

Fecundity and Fertility. One male and one female each from a treatment combination were placed in a small wire-mesh cage, which had a diameter of 8.9 cm and height of 7 cm. Wax paper was placed on the top of the mesh cage as an oviposition site. A glass petri dish held the wax paper in place. The cages were kept on trays covered with water-logged cotton in an environmentally controlled room maintained at 27°C during the day and 16°C during the night with a photoperiod of 16:8 (L:D) h. The experimental unit was a breeding pair in the wire mesh cage and was replicated nine to 34 times. The initial experiment studied adults from the 0.2, 0.1, and 0.05% CLA or safflower oil treatment groups and the control group, for a total of seven treatment combinations. A follow-up experiment required large scale rearing of larvae with the 0.6 and 0.4% CLA diets, and the adults were evaluated along with adults fed the control diet as a larva, for a total of three treatment combinations. The experimental unit

Table 1. Effects of dietary treatment concentration on time to pupal stage, time to adult emergence, and pupal weight of *O. nubilalis*[a]

Variable	Dietary treatment concentration (%)						*F*	df	*P*
	Control	0.05	0.1	0.2	0.4	0.6			
Time to pupal stage (d)									
CLA	14.6 ± 0.1a	15.4 ± 0.1b	15.1 ± 0.1b	15.8 ± 0.1c	17.1 ± 0.2d	18.1 ± 0.2e	62.79	5,359	<0.0001
SAF	14.5 ± 0.1a	15.2 ± 0.1b	N.T.	N.T.	15.1 ± 0.1b	16.0 ± 0.1c	20.14	3,266	<0.0001
Pupal wt (mg)									
CLA	94 ± 2a	105 ± 2a	102 ± 2a	100 ± 2a	95 ± 2a	96 ± 2a	2.83	5,359	0.0159
SAF	101 ± 2a	101 ± 2a	N.T.	N.T.	102 ± 2a	94 ± 3a	1.75	3,266	0.1581
Time to adult emergence (d)									
CLA	22.1 ± 0.1a	22.6 ± 0.1a	22.3 ± 0.2a	22.8 ± 0.2a	24.1 ± 0.2b	24.3 ± 0.6b	13.42	5,173	<0.0001
SAF	22.1 ± 0.1a	22.3 ± 0.1ab	N.T.	N.T.	22.5 ± 0.1b	22.9 ± 0.1c	7.41	3,186	0.0001

CLA, conjugated linoleic acid; N.T., not tested; SAF, safflower oil.

[a] Values are reported as LSM ± SEM. Means within a row followed by the same letter are not different ($P < 0.05$; Student-Newman-Keuls test).

(the breeding pair in the cage) was replicated six to 13 times. The pairs were kept together for 7 d. Eggs on the wax paper were collected each morning, and a new wax paper was put in its place. At the end of the week, the adults were destroyed. Individual eggs were counted for each pair by using a light microscope. Fertile eggs could be distinguished from infertile eggs as they developed by using the microscope. The percentage of pairs to produce fertile eggs also was recorded.

Statistical Analyses. The time (days) to pupa, pupal mass (milligrams), time (days) to adult emergence, total lipid content, and FAME composition were analyzed by using analysis of variance (ANOVA), and the means compared by the Student-Newman-Keuls test (PROC GLM, SAS Institute 2002). Data of mortality at pupal and adult stages were corrected for control mortality (Hoekstra 1987), and they were used to calculate LC_{50} values for *O. nubilalis* fed the CLA or safflower oil diets as larvae on a logarithmic base 10 scale (PROC Probit, SAS Institute 2002). The slopes and intercepts of the concentration-response curves, on a logarithmic base 10 scale, were compared using the SAS procedure PROC GLIMMIX (SAS Institute 2002). The data of percentage of survivors to the pupal and adult stages were normalized by using arcsine square-root transformation before ANOVA, and means were separated by using the Student-Newman-Keuls test (PROC GLM, SAS Institute 2002). The percentage of fertile eggs produced (by adults that produced at least one egg) data were transformed by using arcsine square-root transformation before ANOVA and analyzed by the LSMEANS option of PROC GLM with Tukey adjustment for pair-wise comparisons among treatment combinations (SAS Institute 2002). For the initial experiment, fecundity for each of the seven treatment combinations was analyzed by using the chi-square test in a 7 by 2 contingency table for independence between treatment combination and the production of at least one fertile egg (CHISQ option, PROC FREQ, SAS Institute 2002). This test was not valid, however, because some of the cells had expected counts less than five. Therefore, pairwise comparisons of interest were preformed by using Fisher exact test and the Bonferonni method to adjust *P* values (EXACT option, PROC FREQ, SAS Institute 2002). For the follow-up experiment, fecundity for each of the three treatment combinations also was analyzed by pairwise comparisons of interest by using Fisher exact test and the Bonferonni method to adjust *P* values (EXACT option, PROC FREQ, SAS Institute 2002). The percentage of fertile eggs produced data were transformed by using arcsine square-root transformation before ANOVA and analyzed by the LSMEANS option of PROC GLM with Tukey adjustment for pairwise comparisons among treatment combinations (SAS Institute 2002). Adults were considered fecund if at least one fertile egg was pro-

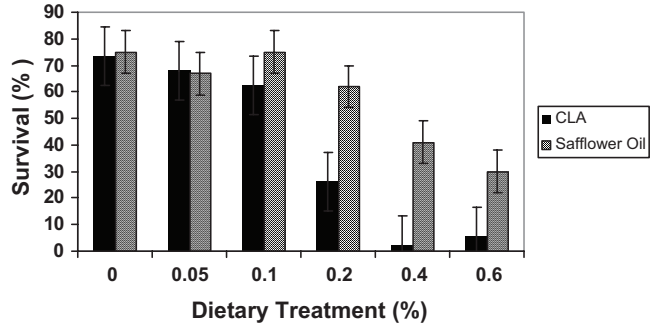

Fig. 1. Survival of *O. nubilalis* neonates to the pupal stage fed different concentrations of CLA or safflower oil in the meridic diet.

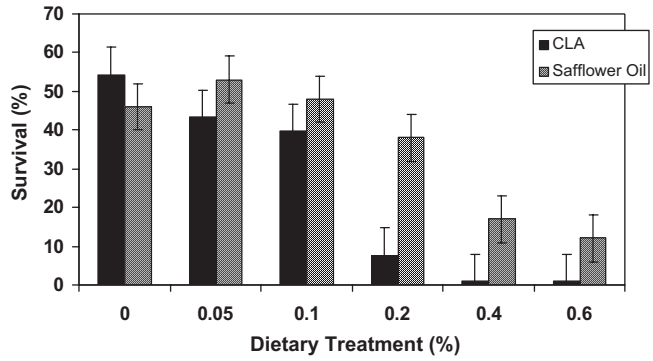

Fig. 2. Survival of *O. nubilalis* neonates to the adult stage fed different concentrations of CLA or safflower oil in the meridic diet as a larva.

duced. Nontransformed data are presented in figures and tables.

Results

Impact of Dietary CLA on Development. Dietary CLA increased the time (days) required for development from the neonate to the pupal stage and to the adult stage (Table 1). Dietary safflower oil also increased the time (days) required for development from the neonate to the pupal stage and to the adult stage (Table 1). Feeding either CLA or safflower oil increased time to adult emergence. The developmental time increase, however, was more pronounced for *O. nubilalis* fed the CLA diets. Pupal weight (milligrams) was not affected by dietary CLA or safflower oil. The pupae were not sexed before weighing; thus, means for each treatment combination included weights of both males and females.

Impact of Dietary CLA on Survival. Dietary CLA decreased the percentage of *O. nubilalis* neonates surviving to the pupal stage ($F = 8.63$; df = 5, 12; $P < 0.0011$) and to the adult stage ($F = 19.72$; df = 5, 12; $P < 0.0001$) (Figs. 1 and 2, respectively). The LC_{50} values for pupae and adults were 0.14% CLA and 0.10% CLA, respectively (Table 2). Dietary safflower oil also decreased the percentage of surviving *O. nubilalis* neonates to the pupal stage ($F = 5.49$; df = 5, 12; $P = 0.0074$) and to the adult stage ($F = 9.02$; df = 5, 12; $P = 0.0009$) (Figs. 1 and 2, respectively). The LC_{50} values for pupae and adults were 0.55% safflower oil and 0.34% safflower oil, respectively (Table 2). Significant differences were found between the slopes of the CLA and safflower oil concentration-response curves at the pupal stage ($F = 28.38$; df = 1, 26; $P < 0.0001$) and the adult stage ($F = 11.23$; df = 1, 26; $P = 0.0025$). The intercepts of the curves also were significantly different at the pupal stage ($F = 23.14$; df = 1, 8.107; $P = 0.0013$) and the adult stage ($F = 19.58$; df = 1, 26; $P = 0.0002$).

Table 2. Toxicity of CLA and safflower oil to *O. nubilalis* at the pupal and adult stages

Treatment and stage of development	*n*	Slope (SEM)[a]	LC_{50} (95% FL)[b]	df	χ^2[c]
CLA pupae	18	2.7 (0.60)	0.14 (0.09–0.22)	1	20.01
CLA adults	18	2.9 (0.55)	0.10 (0.07–0.14)	1	28.14
Safflower oil pupae	18	1.5 (0.56)	0.55 (0.26–50)	1	6.71
Safflower oil adults	18	2.6 (0.52)	0.34 (0.25–0.52)	1	24.66

FL, fiducial limit.
[a] Corrected for control mortality using Abbott's formula.
[b] Units are percentage of CLA or safflower oil in meridic diet (calculated as weight to volume).
[c] $P < 0.001$ *for all* χ^2 *values.*

Impact of Dietary CLA on Fatty Acid Profile. The fatty acid profiles of pupae fed a CLA-enriched diet as larvae (CLA pupae) were significantly different compared with that of the pupae that had been fed the control diet during their larval stage (control pupae) (Table 3). Palmitic (16:0), palmitoleic (16:1), oleic (18:1) and linoleic (18:2n-6) acids made up the majority of fatty acids in *O. nubilalis* control pupae. In CLA pupae, the concentration of saturated fatty acids (SFA) increased, whereas the concentration of monounsaturated fatty acids (MUFA) decreased. The value of the Δ9-desaturase index decreased as dietary CLA concentration increased, indicating the inhibition of the Δ9-desaturase by CLA. The *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers were incorporated into the tissues at the expense of other polyunsaturated fatty acids (PUFA). The SFA include 14:0, 16:0, and 18:0; the MUFA include 16:1and 18:1; the PUFA include 18:2, 18:3, 20:5, and the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers. As dietary CLA concentration increased, the concentrations of the 18:2n-6 and linolenic (18:3n3) acids decreased. Total lipid content was not affected by dietary CLA. Total SFA ($F = 1.40$; df = 5, 12; $P = 0.29$) and MUFA ($F = 0.97$; df = 5, 12; $P = 0.47$) concentrations did not change in safflower oil pupae. Within the MUFA, 18:1 increased ($F = 32.01$; df = 5, 12; $P < 0.0001$), whereas 16:1 decreased (13.12; df = 5, 12; $P = 0.0002$). This response probably was caused by the safflower oil, which is rich in 18:1. The value of the Δ9-desaturase index did not change with increased concentration of safflower oil in the diet ($F = 1.17$; df = 5, 12; $P = 0.3765$). Interestingly, 18:3n-3 decreased from 1.2% of total fatty acids in controls to 0.8% of total fatty acids in pupae fed the

Table 3. Effects of dietary CLA concentration on fatty acid composition, total lipid content, and Δ 9-desaturation on *O. nubilalis* pupae[a]

Variable	Dietary CLA concentration (%)						*F*	df	*P*
	Control	0.05	0.1	0.2	0.4	0.6			
Fatty acid[b]									
14:0	0.41 ± 0.02a	0.42 ± 0.02a	0.38 ± 0.02a	0.50 ± 0.02b	0.54 ± 0.02b	0.51 ± 0.02b	11.20	5, 10	0.0008
16:0	28 ± 0.57a	34 ± 0.70b	37 ± 0.57c	39 ± 0.57c	42 ± 0.57d	42 ± 0.70d	83.39	5, 10	<0.0001
16:1n7	33 ± 0.76a	28 ± 0.93b	23 ± 0.76c	20 ± 0.76d	15 ± 0.76e	12 ± 0.93e	96.27	5, 10	<0.0001
18:0	1.3 ± 0.12a	1.9 ± 0.15b	2.5 ± 0.12c	3.0 ± 0.12d	4.0 ± 0.12e	4.7 ± 0.15f	88.56	5, 10	<0.0001
18:1n9	25 ± 0.55a	24 ± 0.68a	23 ± 0.55ab	22 ± 0.55bc	19 ± 0.55d	20 ± 0.67cd	15.34	5, 10	0.0002
18:2n6	10 ± 0.24a	9.2 ± 0.30ab	9.7 ± 0.24ab	9.6 ± 0.24ab	8.7 ± 0.24b	7.6 ± 0.30c	10.49	5, 10	0.0010
18:3n3	1.2 ± 0.03a	1.1 ± 0.03b	1.1 ± 0.03ab	1.0 ± 0.03b	0.84 ± 0.03c	0.67 ± 0.03d	40.64	5, 10	<0.0001
c9,t11 CLA	N.D.a	0.97 ± 0.15a	1.9 ± 0.12b	3.1 ± 0.12c	5.5 ± 0.12d	6.9 ± 0.15e	395.54	5, 10	<0.0001
t10,c12 CLA	N.D.a	0.60 ± 0.14a	1.3 ± 0.11b	2.3 ± 0.11c	4.1 ± 0.11d	4.9 ± 0.14e	245.56	5, 10	<0.0001
20:5n3	0.51 ± 0.19a	N.D.a	N.D.a	0.06 ± 0.19a	0.06 ± 0.19a	N.D.a	1.04	5, 10	0.4476
SFA	30 ± 0.64a	36 ± 0.79b	40 ± 0.64c	42 ± 0.64d	47 ± 0.64e	48 ± 0.79e	99.08	5, 10	<0.0001
MUFA	58 ± 0.73a	52 ± 0.90b	46 ± 0.73c	42 ± 0.73d	34 ± 0.73e	32 ± 0.89e	170.40	5, 10	<0.0001
PUFA	12 ± 0.50a	12 ± 0.61a	14 ± 0.50a	16 ± 0.50b	19 ± 0.50c	20 ± 0.61d	42.53	5, 10	<0.0001
Total lipids[c]	169 ± 15a	190 ± 15a	183 ± 15a	170 ± 15a	174 ± 15a	153 ± 18a	0.65	5, 11	0.6656
Δ9-Desaturase Index[d]	67 ± 0.73a	59 ± 0.90b	54 ± 0.73c	50 ± 0.73d	42 ± 0.73e	41 ± 0.90e	161.31	5, 10	<0.0001

Means within a row followed by the same letter are not different ($P < 0.05$; Student-Newman-Keuls test). N.D., not detected; SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids, including CLA.

[a] Values reported as LSM ± SEM. Means are average of two or three replicates of five pooled pupae.

[b] Weight percentage of fatty acid methyl esters.

[c] micrograms per milligram of tissue.

[d] Calculated as 100 × [(16:1n-7 + 18:1n-9)/(16:1n-7 + 18:1n-9 + 16:0 + 18:0)].

0.60% safflower oil diet ($F = 9.02$; df = 5, 12; $P = 0.0009$). 18:2n-6 did not decrease ($F = 1.05$; df = 5, 12; $P = 0.4310$). Total lipid content was not affected by dietary safflower oil ($F = 2.65$; df = 5, 12; $P = 0.0773$).

Fatty acid profiles of adult females and males reared on the CLA-enriched diets also were different from those of the controls (Tables 4 and 5, respectively); 16:0, 16:1, 18:1, and 18:2n-6 made up the majority of fatty acids in adults fed the control diet as larvae. 18:3n-3 was decreased from 1.2% in control pupae to 0.9 and 0.8% in control adult females and males, respectively. The concentration of SFA increased, whereas the concentration of MUFA decreased with increased concentration of CLA in the diet. The value of the Δ9-desaturase index decreased as dietary CLA concentration increased, with the decline being sharper in males compared with females. Again, the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers were incorporated into the tissues. Gender differences were observed: females had higher concentrations of

Table 4. Effect of dietary CLA concentration on fatty acid composition, total lipid content, and Δ9-desaturation on *O. nubilalis* adult females[a]

Variable	Dietary CLA concentration (%)				*F*	df	*P*
	Control	0.05	0.1	0.2			
Fatty acid[b]							
14:0	0.38 ± 0.06a	0.43 ± 0.06a	0.11 ± 0.06b	N.D.b	13.28	3, 8	0.0018
16:0	30 ± 0.61a	37 ± 0.61b	35 ± 0.61b	41 ± 0.61c	54.70	3, 8	<0.0001
16:1n7	31 ± 0.68a	26 ± 0.68b	27 ± 0.68b	22 ± 0.68c	33.36	3, 8	<0.0001
18:0	1.4 ± 0.11a	2.0 ± 0.11b	2.1 ± 0.11b	2.3 ± 0.11b	14.10	3, 8	0.0015
18:1n9	25 ± 0.68a	24 ± 0.68ab	24 ± 0.68ab	21 ± 0.68b	4.94	3, 8	0.0316
18:2n6	11 ± 0.37a	9.4 ± 0.37b	8.9 ± 0.37b	9.0 ± 0.37b	7.41	3, 8	0.0107
18:3n3	0.88 ± 0.03a	0.68 ± 0.03b	0.44 ± 0.03c	N.D.d	180.97	3, 8	<0.0001
c9,t11 CLA	N.D.a	0.81 ± 0.04b	1.5 ± 0.04c	2.8 ± 0.04d	726.02	3, 8	<0.0001
t10,c12 CLA	N.D.a	0.50 ± 0.05b	1.1 ± 0.05c	1.9 ± 0.05d	273.96	3, 8	<0.0001
20:5n3	0.09 ± 0.01a	0.12 ± 0.01a	N.D.b	N.D.b	24.49	3, 8	0.0002
SFA	32 ± 0.61a	39 ± 0.61b	37 ± 0.61b	43 ± 0.61c	60.47	3, 8	<0.0001
MUFA	56 ± 0.78a	50 ± 0.78b	51 ± 0.78b	43 ± 0.78c	47.90	3, 8	<0.0001
PUFA	12 ± 0.48ab	11 ± 0.48a	12 ± 0.48ab	14 ± 0.48b	4.38	3, 8	0.0421
Total lipids[c]	163 ± 9.9a	169 ± 9.9a	171 ± 9.9a	204 ± 9.9a	3.49	3, 8	0.0699
Δ9-desaturase Index[d]	64 ± 0.78a	56 ± 0.77b	58 ± 0.77b	50 ± 0.77c	58.54	3, 8	<0.0001

Means within a row followed by the same letter are not different ($P < 0.05$; Student-Newman-Keuls test). N.D., not detected; SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids, including CLA.

[a] Values reported as LSM ± SEM. Means are average of three replicates of five pooled adults.

[b] Weight percentage of fatty acid methyl esters.

[c] Micrograms per milligram of tissue.

[d] Calculated as 100 × [(16:1n-7 + 18:1n-9)/(16:1n-7 + 18:1n-9 + 16:0 + 18:0)].

Table 5. Effect of dietary CLA concentration on fatty acid composition, total lipid content, and Δ9-desaturation on *O. nubilalis* adult males[a]

Variable	Dietary CLA concentration (%)				*F*	df	*P*
	Control	0.05	0.1	0.2			
Fatty acid[b]							
14:0	0.41 ± 0.05a	0.44 ± 0.05a	0.48 ± 0.05a	0.10 ± 0.05b	12.19	3, 8	0.0024
16:0	31 ± 0.71a	36 ± 0.71b	40 ± 0.71c	44 ± 0.71d	62.26	3, 8	<0.0001
16:1n7	31 ± 0.52a	26 ± 0.52b	22 ± 0.52c	19 ± 0.52d	91.95	3, 8	<0.0001
18:0	1.4 ± 0.06a	2.0 ± 0.06b	2.5 ± 0.06c	3.1 ± 0.06d	144.10	3, 8	<0.0001
18:1n9	25 ± 0.39a	25 ± 0.39ab	24 ± 0.39bc	23 ± 0.39c	9.43	3, 8	0.0053
18:2n6	10 ± 0.29a	8.9 ± 0.29b	8.1 ± 0.29bc	7.2 ± 0.29c	22.75	3, 8	0.0003
18:3n3	0.79 ± 0.04a	0.57 ± 0.04b	0.53 ± 0.04b	N.D.c	91.32	3, 8	<0.0001
c9,t11 CLA	N.D.a	0.71 ± 0.04b	1.3 ± 0.04c	2.6 ± 0.04d	859.73	3, 8	<0.0001
t10,c12 CLA	N.D.a	0.42 ± 0.04b	0.82 ± 0.04c	1.7 ± 0.04d	318.22	3, 8	<0.0001
20:5n3	0.15 ± 0.01a	0.12 ± 0.01b	0.12 ± 0.01b	N.D.c	82.04	3, 8	<0.0001
SFA	33 ± 0.68a	38 ± 0.68b	43 ± 0.68c	47 ± 0.68d	81.85	3, 8	<0.0001
MUFA	56 ± 0.57a	51 ± 0.57b	46 ± 0.57c	42 ± 0.57d	119.06	3, 8	<0.0001
PUFA	11 ± 0.30a	11 ± 0.30a	11 ± 0.30a	11 ± 0.30a	1.44	3, 8	0.3017
Total lipids[c]	205 ± 26a	220 ± 26a	221 ± 26a	272 ± 26a	1.25	3, 8	0.3534
Δ9-desaturase Index[d]	64 ± 0.72a	58 ± 0.72b	52 ± 0.72c	47 ± 0.72d	96.17	3, 8	<0.0001

Means within a row followed by the same letter are not different ($P < 0.05$; Student-Newman-Keuls test). N.D., not detected; SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids, including CLA.

[a] Values reported as LSM ± SEM. Means are average of three replicates of five pooled adults.

[b] Weight percentage of fatty acid methyl esters.

[c] Micrograms per milligram of tissue.

[d] Calculated as 100 × [(16:1n-7 + 18:1n-9)/(16:1n-7 + 18:1n-9 + 16:0 + 18:0)].

both CLA isomers than did the males. Both 18:3n-3 and 18:2n-6 decreased with increasing dietary CLA concentration. No 18:2n-6 acid was detected in adults fed the 0.20% CLA diet as larvae. Total lipid content was not affected.

Adult females and males reared on the safflower oil-enriched diets did not differ from control females and males in total SFA or MUFA concentrations. As was observed in the pupae, the adults reared on the safflower oil diets had slightly increased 18:1n-9 (females: $F = 3.01$; df = 3, 8; $P = 0.0944$, males: $F = 6.07$; df = 3, 8; $P = 0.0186$); however, no apparent decrease in 16:1n-7 was observed. The value of the Δ9-desaturase index did not change with increased concentration of safflower oil in the diet. The concentration of 18:3n-3 was decreased slightly in females ($F = 4.50$; df = 3, 8; $P = 0.0394$) and males ($F = 5.87$; df = 3, 8; $P = 0.0203$), whereas the 18:2n-6 acid remained unchanged compared with those of the control adults. Total lipid content was not affected.

The fatty acid profiles of eggs from adults fed a CLA diet as larvae (CLA eggs) differed from the fatty acid

Table 6. Effect of dietary CLA concentration on fatty acid composition, total lipid content, and Δ 9-desaturation of *O. nubilalis* eggs[a]

Variable	Dietary CLA concentration (%)				*F*	df	*P*
	Control	0.05	0.1	0.2			
Fatty acid[b]							
14:0	0.23 ± 0.01a	0.25 ± 0.01ab	0.29 ± 0.01b	0.30 ± 0.01b	5.36	3, 8	0.0257
16:0	31 ± 0.25a	33 ± 0.25b	35 ± 0.25c	37 ± 0.25d	108.32	3, 8	<0.0001
16:1n7	27 ± 0.48a	24 ± 0.48b	22 ± 0.48c	18 ± 0.48d	66.69	3, 8	<0.0001
18:0	2.7 ± 0.07a	3.0 ± 0.07b	3.3 ± 0.07c	4.1 ± 0.07d	67.50	3, 8	<0.0001
18:1n9	29 ± 0.21a	28 ± 0.21a	26 ± 0.21b	25 ± 0.21c	68.21	3, 8	<0.0001
18:2n6	9.0 ± 0.21a	9.5 ± 0.21a	9.1 ± 0.21a	9.0 ± 0.21a	1.09	3, 8	0.4074
18:3n3	0.83 ± 0.14a	0.57 ± 0.14a	0.74 ± 0.14a	0.76 ± 0.14a	0.68	3, 8	0.5862
c9,t11 CLA	N.D.a	1.1 ± 0.08b	2.1 ± 0.08c	3.8 ± 0.08d	360.10	3, 8	<0.0001
t10,c12 CLA	N.D.a	0.56 ± 0.06b	1.2 ± 0.06c	2.2 ± 0.06d	248.13	3, 8	<0.0001
20:5n3	0.27 ± 0.04a	0.24 ± 0.04a	0.25 ± 0.04a	0.17 ± 0.04a	1.06	3, 8	0.4181
SFA	34 ± 0.24a	36 ± 0.24b	39 ± 0.24c	41 ± 0.24d	183.56	3, 8	<0.0001
MUFA	56 ± 0.47a	51 ± 0.47b	48 ± 0.47c	42 ± 0.47d	143.59	3, 8	<0.0001
PUFA	10 ± 0.41a	12 ± 0.41b	13 ± 0.41c	16 ± 0.41d	36.10	3, 8	<0.0001
Total lipids[c]	90.1 ± 17a	134 ± 17a	138 ± 17a	163 ± 17a	3.02	3, 8	0.0941
Δ9-desaturase Index[d]	62 ± 0.35a	59 ± 0.35b	55 ± 0.35c	51 ± 0.35d	191.44	3, 8	<0.0001

Means within a row followed by the same letter are not different ($P < 0.05$; Student-Newman-Keuls test). N.D., not detected; SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids, including CLA.

[a] Values reported as LSM ± SEM. Means are average of three replicates of 500-mg egg masses.

[b] Weight percentage of fatty acid methyl esters.

[c] Micrograms per milligram of tissue.

[d] Calculated as 100 × [(16:1n-7 + 18:1n-9)/(16:1n-7 + 18:1n-9 + 16:0 + 18:0)].

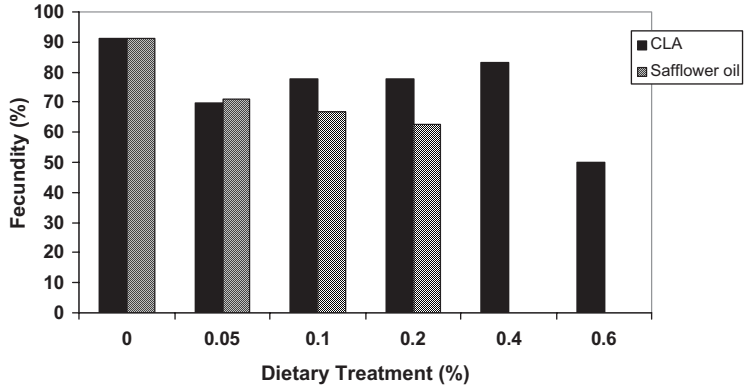

Fig. 3. Percentage of pairs that oviposited eggs as a function of treatment concentration in the diet.

profile of eggs from adults fed the control diet as larvae (control eggs) (Table 6). In CLA eggs, the concentration of total SFA increased whereas the concentration of total MUFA decreased with increased CLA concentration in the diet of the parents. The value of the Δ9-desaturase index decreased as parent dietary CLA concentration increased. The *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers were incorporated into the egg lipids, indicating these fatty acids were transferred from the female to the eggs. The major PUFA, 18:2n6 and 18:3n3, did not differ among treatment combinations. Total lipid content was not affected.

Impact of Dietary CLA on Fecundity and Fertility. In both the initial experiment and the follow-up experiment to evaluate the effects of the 0.40 and 0.60% CLA diets, the results of Fisher exact test for each of the pairwise comparisons indicated fecundity did not differ among treatment combinations (Fig. 3). No treatment effects on the percentage of fertile eggs laid were observed in the initial experiment ($F = 0.72$; df = 6, 116; $P = 0.6381$) or in the follow-up experiment ($F = 2.69$; df = 2, 27; $P = 0.0857$) (Fig. 4). Only 50% of the breeding pairs in the 0.60% CLA treatment group produced any eggs. At higher dietary CLA concentrations, fecundity may be significantly impacted.

Discussion

The results presented here support our hypothesis that dietary CLA alters the fatty acid profile of *O. nubilalis.* Our data clearly demonstrate the increase in SFA concentration, namely, 14:0, 16:0, and 18:0, with a decrease in MUFA concentration, namely, 16:1n-7 and 18:1n-9, as a function of increasing dietary CLA. The Δ9-desaturase index decreased as dietary CLA concentration increased, but it remained unchanged in insects fed the safflower oil-enriched diets. These data are consistent with the inhibition of Δ9-desaturase by CLA. CLA inhibition of Δ9-desaturase has been demonstrated in several animal models, including mammals and poultry, and human breast cancer cell systems (Lee et al. 1998, Choi et al. 2002, Shang et al. 2005). Because fatty acids are precursors to cuticle hydrocarbons, pheromones, and other longer-chain, more unsaturated fatty acids, alterations to the fatty acid composition may have severe implications in many biochemical processes.

In general, diets high in PUFA lead to higher percentages of tissue PUFA and lower percentages of tissue MUFA (Stanley-Samuelson et al., 1988). Our data show a concentration-dependent increase in tis-

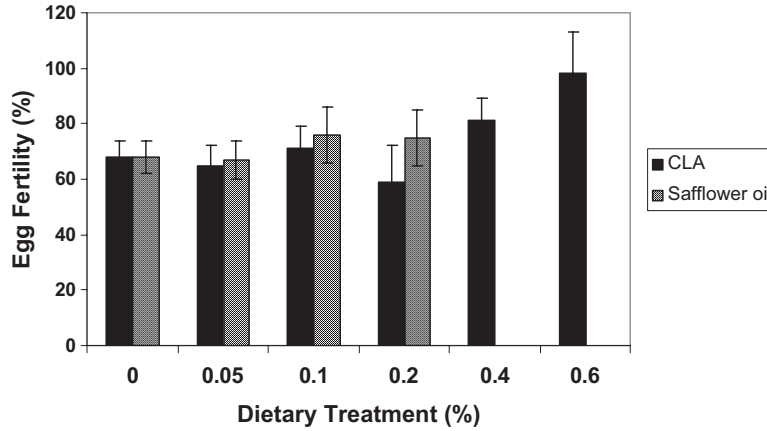

Fig. 4. Percentage of fertile eggs as a function of treatment concentration in the diet.

sue CLA whereas the other PUFA, namely, 18:2n-6 and 18:3n-3, decreased. We believe the decrease in these two PUFA may be because of a competitive inhibition in uptake by the midgut caused by the high concentration of CLA in the diet. Only one 20-carbon PUFA was detected, which was at a relatively low concentration in both pupae and adults. Because we used whole body lipid extracts, we may have been unable to detect other long-chain PUFA that may be in very low concentrations in select tissues and lipid classes.

Our results indicate that CLA is incorporated into the tissue lipids of *O. nubilalis* with adverse effects on development and survival. The time required for development was increased, and survival was decreased. Although dietary safflower oil also decreased survival, the impact of CLA on survival was more severe. The decrease in survival observed in the insects fed the safflower oil diets may have been caused by the increased lipid content as compared with that of the optimal standard meridic diet. We believe the altered fatty acid profiles may contribute to the increased developmental time and decreased survival. Dietary CLA decreased the Δ9-desaturation index, whereas dietary safflower oil did not affect this index. This difference between CLA and safflower oil may explain the differing effects on survival and rate of development. Our results are in contrast to the results of Park et al. (2000) that showed CLA being incorporated into the house fly with no adverse effects. The fatty acid profile, however, was not reported in that study. It would be of interest to know whether CLA inhibits Δ9-desaturation in the house fly. Mammals and poultry fed CLA-enriched diets also incorporate CLA isomers into tissue lipids (Thiel-Cooper et al. 2001, Yang et al. 2003). Survival of mammals and poultry fed CLA-enriched diets is not affected adversely. Rats fed a 1% CLA diet for 18 mo did not differ in weight gain or survival rate compared with rats fed the control diet (Park et al. 2005).

We did not find adverse effects on reproduction, which is consistent with the results of Park et al. (2000). This result is in contrast to the results of several poultry studies in which dietary CLA caused complete embryo mortality or decreased hatchability (Aydin et al. 2001, Aydin and Cook 2004). The decreased concentration of 18:1n-9 in the yolk was believed to be a major factor in embryo mortality. We did find altered fatty acid profiles in the eggs of adults fed CLA-enriched diets as larvae. The *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers were incorporated into the lipids of the eggs, total SFA increased, and total MUFA decreased as the concentration of CLA in the diet of the parents increased. We determined the fertility of the *O. nubilalis* eggs before hatching, when the black larval head capsule was clearly visible. We cannot be sure that the neonates would have survived through all life stages. The fatty acid profile alterations may have adverse effects on the survival and development of the neonate.

Our results may have implications in future development of biorational pest control agents. Very few insecticides currently on the market target lipid metabolism. The tetronic acid insecticides are the only known insecticides that interfere with lipid biosynthesis. CLA significantly decreases the survival of *O. nubilalis* and affects the biochemistry of the insect with a novel mode of action.

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