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Influence of Larval Diet and Adult Age on the Chemical Composition of Female Pheromone Glands of *Copitarsia decolora* (Lepidoptera: Noctuidae): Implications for Semiochemical-Based Crop Protection

Luis Díaz ¹, Daniel Rodríguez ^{2,*} and Ericsson Coy-Barrera ³

¹ Bioprospecting Research Group, School of Engineering, Universidad de La Sabana, Chía 140013, Colombia; luis.diaz1@unisabana.edu.co

² Biological Control Laboratory, Faculty of Basic and Applied Science, Universidad Militar Nueva Granada, Cajicá 250247, Colombia

³ Bioorganic Chemistry Laboratory, Faculty of Basic and Applied Science, Universidad Militar Nueva Granada, Cajicá 250247, Colombia; ericsson.coy@unimilitar.edu.co

* Correspondence: daniel.rodriguez@unimilitar.edu.co

Abstract

Copitarsia decolora (Guenée) is a polyphagous pest of significant agricultural importance in the Americas, yet its nutritional and pheromone-related variations remain to be understood. This study evaluated the effects of larval diet and female adult age on life-cycle parameters, fertility, survival, and pheromone gland composition in *C. decolora* reared on *Alstroemeria* leaves (primary host), cauliflower florets (secondary host), and an artificial diet. While the overall life-cycle duration was similar among diets, *Alstroemeria*-fed larvae showed the highest fertility and adult longevity. Diet strongly influenced pheromone gland chemistry, with multivariate and quantitative analyses revealing significant diet- and age-dependent variations in key pheromone components, including (Z)-tetradec-9-enyl acetate (Z9-14:Ac) and (Z)-tetradec-9-en-1-ol (Z9-14:OH). Females reared on *Alstroemeria* exhibited enhanced pheromone production, whereas artificial diets favored higher alkane accumulation. These findings demonstrate nutritional modulation of pheromone biosynthesis and highlight the importance of diet standardization in insect rearing and semiochemical-based pest management strategies.

Keywords: lepidoptera; moths; *Copitarsia*; reproduction; pheromone gland; diet

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1. Introduction

The genus *Copitarsia*, particularly *Copitarsia decolora* (Guenée) (Lepidoptera: Noctuidae), is recognized as an important agricultural pest due to its polyphagous nature and broad host range, affecting both horticultural and field crops across the Americas [1]. It is distributed from Mexico to Argentina and other parts of South America, with a presence recorded in countries such as Colombia, Ecuador, Venezuela, Peru, and Chile [2]. This noctuid moth infests a variety of economically significant plants, including cruciferous vegetables such as cabbage, broccoli, and cauliflower, as well as asparagus and ornamental species like cut flowers [3,4]. The larval stage causes the most significant damage, with voracious feeding leading to yield losses, reduced crop quality, and substantial economic

impacts [3,5]. Particularly for export-oriented crops, infestations can trigger strict quarantine measures, including shipment rejections or incineration, in importing countries such as the United States and the Netherlands, resulting in additional economic consequences [6,7].

C. decolora presents a cryptic taxonomic status, as it is often misidentified or associated with other *Copitarsia* species. In fact, the so-called *C. decolora* complex encompasses a group of four closely related and economically important moth species, namely *C. decolora*, *C. corruda* (Pogue & Simmons), *C. incommode* (Walker), and *C. gibberosa* (Pogue) [8]. In Colombia, *C. decolora* is associated with cut flower crops cultivated on the Bogotá Plateau [9]. Evidence also suggests the coexistence of *C. decolora* and *C. uncinata* (Burgos & Leiva) in certain *Alstroemeria* plantations. The latter exhibits similar biological traits, such as comparable durations of immature developmental stages, but differs in adult genital morphology, particularly in the male uncus structure [10].

Historically, management of *C. decolora* has relied primarily on organophosphate, carbamate, and pyrethroid insecticides [8]. Although these chemicals provide short-term control, they have also induced resistance, contaminated the environment, and disrupted non-target organisms, including natural enemies [11]. The growing awareness of these limitations has stimulated the development of more sustainable alternatives, particularly integrated pest management (IPM) programs. Among these, semiochemical-based strategies, such as pheromone traps, have gained increasing attention for their specificity, safety, and compatibility with IPM principles. Studies have identified (Z)-tetradec-9-enyl acetate (Z9-14:Ac) and (Z)-tetradec-9-enol (Z9-14:OH) as the major components of the *C. decolora* sex pheromone, with optimized blends proving effective for male attraction and population suppression [12,13]. Such pheromone-based systems not only enhance monitoring and mass trapping but also improve decision-making in pest control interventions, promoting environmentally sound crop protection [14].

The success of semiochemical-based management depends on a comprehensive understanding of the insect's biology, ecology, and chemical communication [15]. *C. decolora* exhibits remarkable phenotypic plasticity, with its developmental rate, fecundity, and longevity influenced by environmental conditions such as temperature, photoperiod, and diet [5,16]. Among these, diet plays a pivotal role in shaping larval performance and adult reproductive potential. Larvae reared on artificial diets often display lower survival and fecundity than those fed on natural host plants, such as cauliflower or broccoli [17]. Host plant composition not only provides essential nutrients but can also alter the chemical profile of sex pheromone glands, thereby influencing mating behavior and reproductive success [18]. In addition to chemical control and semiochemicals, biological control agents, especially egg parasitoids such as *Trichogramma pretiosum* (Riley) (Hymenoptera; Trichogrammatidae) and *T. atopovirilia* (Oatman & Platner), have shown promise in reducing egg survival, providing a complementary and ecologically compatible approach [19]. Their performance can be enhanced through habitat management, such as increasing floral diversity to provide nectar and refuge, which strengthens parasitoid establishment and persistence in the field [20].

Developing efficient semiochemical-based control strategies requires a sequential research approach, encompassing compound identification, synthesis, dose optimization, and trap design. Such studies rely on well-established insect rearing systems to ensure a consistent supply of healthy specimens for experimental assays [21]. Laboratory rearing provides the necessary biological material while minimizing mortality, thus enabling controlled investigations on genetics, physiology, and chemical ecology [22,23]. The nutritional composition of rearing diets strongly affects insect growth rate, fertility, sex ratio, and survivorship [22,24].

Natural host plant diets provide optimal nutrition but are costly and difficult to standardize for mass rearing, whereas artificial diets, prepared with defined ingredients, reduce costs and contamination risks [25]. Artificial diets are classified as holidic (chemically defined), meridic (partly defined), or oligidic (based on natural ingredients) [26]. Oligidic diets have been successfully used to rear *C. incommoda* and *C. consueta* (Walker) larvae [27,28], supporting the pheromone-based monitoring system development [12,13].

Reproductive performance and pheromone communication in Lepidoptera are strongly modulated by nutritional and physiological factors, which affect energy allocation, endocrine regulation, morpho-functional aspects, behavior, and lipid metabolism [29]. Dietary inputs, particularly during larval development, have been shown to influence the biosynthesis and composition of pheromone blends in noctuids and bombycids by altering lipid droplet formation and precursor availability in pheromone glands [30–32]. For instance, variations in larval diet can modify the chemical composition and pheromone titers in *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and *S. exigua* (Hübner), affecting reproductive success and mate communication [33,34]. Despite this growing evidence, the influence of both larval and adult diets on the reproductive physiology and glandular chemistry of *C. decolora* remains largely unexplored. Moreover, while most studies have focused on identifying major pheromone components, little attention has been given to the broader chemical diversity of the female pheromone gland across adult maturation. Previous findings indicate that the mating age of males and females affects the reproductive potential and longevity of *C. decolora* [16]. Based on this, it could be expected that the composition of the sex pheromone gland would show variations due to age. If this is the case, it is also pertinent to associate these compositional variations with changes in the life history parameters as a function of age. Recent research also indicates that male nutrition influences pheromone-mediated behaviors and sexual signaling [35,36], underscoring the importance of considering both developmental and adult nutritional inputs when examining semiochemical systems.

Under this context, this study investigates how larval diet (natural host versus artificial) and adult age affect the chemical composition of the female pheromone glands in *C. decolora*. By integrating nutritional and physiological perspectives, this study aims to explore the patterns underlying the gland chemistry and pheromone accumulation in this pest species. It also seeks to provide insights into theoretical ecological frameworks for developing diet-optimized, semiochemical-based monitoring and control strategies for crop protection against *C. decolora*. All field and laboratory experiments presented in this study were conducted in 2022.

2. Materials and Methods

2.1. Insect Sampling and Rearing

Eggs and larvae of *Copitarsia decolora* were collected from cut-flower commercial farms located in the municipalities of El Rosal (4°50'55" N 74°16'18" W), Chía (4°53'47" N 74°3'36" W), Faca (4°39'19" N 74°14'41" W), and Sopó (4°49'12" N 73°57'35" W). Specimens were transported to the laboratory and placed in a rearing room maintained under controlled settings (20.8 ± 0.72 °C; 57.4 ± 5.7% RH; 12:12 h light/dark photoperiod, mimicking the conditions of Colombian and equatorial production zones. Upon reaching the adult stage, pairs of insects (1 female × 1 male) were housed in 15 × 6 cm copulation cages to obtain F1 eggs. A subset of male specimens was used for species identification, based on egg structure and genital morphology, the main distinguishing features for *C. decolora* [3]. Voucher specimens (males and females) were preserved in 95% ethanol and deposited in the Entomological Collection of Universidad Militar Nueva Granada (UMNG-CNC-Cop-2022 series) for reference.

F1 individuals were used to avoid the possible effects of life history present in those collected in the field. Eggs from the rearing system ($n = 90$) were individually placed in ½-ounce cylindrical plastic containers. Newly hatched larvae (neonates) were randomly assigned to one of three diet treatments: (1) *Alstroemeria* sp. leaves ($n = 30$), the primary host plant from which the parental individuals were collected, (2) cauliflower florets (*Brassica oleracea* var. *botrytis*) ($n = 30$), a secondary host, and (3) a custom soybean meal-wheatgerm-based artificial diet ($n = 30$), adapted from a previously reported formulation for *C. decolora* [16]. Each container, housing one individual, represented a biological replicate, and the experimental units were arranged in a completely randomized design. Individuals were monitored daily, and the duration of each developmental stage was recorded to estimate the life-cycle span. Pupae were sexed and separated; once adults emerged, they were paired in copulation cages (one female with one male). Once the F1 individuals reached the adult stage, genitalia were analyzed to rule out the possible presence of individuals from other *Copitarsia* species. No evidence of contamination or mixed populations was detected. All F1 adults used in experimental assays were taxonomically verified as *C. decolora* through examination of male genitalia and egg chorion microstructure, diagnostic traits that unambiguously differentiate this species from *C. uncinata* and other congeners. No evidence of contamination or mixed populations was detected among laboratory cohorts. Adult longevity and sex ratios were recorded for each treatment. Female fertility was assessed by counting eggs laid every two days.

2.2. Gland Extract Preparation and Treatments

Individuals from the same cohort ($n = 30$) were reared under the three diet treatments described above. Newly emerged females from each diet were isolated and housed in plastic containers, where they were fed daily with a 1:9 sugar-water solution provided via a cotton ball. Gland extracts were prepared from virgin females of different ages, dissected during the scotophase period corresponding to their previously determined calling behavior (17:00–22:00 h). For dissection, slight pressure was applied to the terminal tip of the abdomen to expose the gland, which was then excised using microscissors. Glands were immersed in vials containing 2 mL of dichloromethane (5 µL/gland) and stored at $-20\text{ }^{\circ}\text{C}$ for eight days until GC-MS analysis [18]. Nine treatments were evaluated using a 3×3 factorial design, accounting for three age groups (1–2 (E1), 3–4 (E2), and 5–6 (E3) days post-emergence) and the three mentioned diet treatments. These age intervals correspond to distinct phases of adult sexual maturation and pheromone activity previously described for *C. decolora* and related noctuids [12,16], encompassing pre-receptive (E1), active mating (E2), and post-peak (E3) periods of calling behavior. For each treatment (age $\times$ diet), three gland extracts were prepared. Three independent biological replicates per treatment were used, consistent with metabolomic standards, ensuring reproducible clustering in multivariate models. Post hoc power analysis ($\alpha = 0.05$, effect size = 0.8) indicated statistical power > 0.85 to detect diet-dependent differences in major pheromone components.

2.3. Gas Chromatography–Mass Spectrometry (GC-MS) Analyses

Gland extracts and pheromone standards (Z9-14:Ac and Z9-14:OH) were analyzed by injecting 1 µL into a gas chromatograph coupled to a mass spectrometer Thermo Scientific TRACE 1300 Single Quadrupole Mass Spectrometer ISQ-LT (Thermo Fisher Scientific, Waltham, MA, USA). A 60 m low-polarity phase column (Rtx-5ms; 0.25 mm internal diameter; 0.25 µm film thickness) was used. The temperature program as follows: injector temperature: $200\text{ }^{\circ}\text{C}$, splitless injection mode (2 mL/min), oven temperature: initially at $100\text{ }^{\circ}\text{C}$ (held for 1 min), a ramp of $5\text{ }^{\circ}\text{C}/\text{min}$ to $250\text{ }^{\circ}\text{C}$ (held for 5 min), and finally at $10\text{ }^{\circ}\text{C}/\text{min}$ to $280\text{ }^{\circ}\text{C}$ (held for 5 min). Helium was used as the carrier gas at a constant flow

rate of 2 mL/min. Sample injection order was randomized before GC–MS analysis. We generated a reproducible, randomized sequence from the sample metadata (diet × age × replicate) using a fixed RNG seed, stratified the sampling to distribute groups across the run, and injected a pooled QC every eight injections, as well as blanks and standards at fixed positions. When runs were performed in blocks, block randomization was used so that each block contained representatives from all groups.

Chemical identification was performed using the Thermo Xcalibur v3.0 Software and the NIST (National Institute of Standards and Technology) mass spectral library. Retention indices were calculated using a homologous paraffin series (C10–C30) under identical GC–MS conditions and compared with literature data. The standards Z9–14:OH and Z9–14:Ac, purchased from Bedoukian Research Inc. (Danbury, CT, USA), with purities of 95% and 96%, respectively, were used as external standards to verify their presence and quantify them in gland extracts (*vide infra*). A feature intensity table (FIT) was generated from GC–MS data through pre-processing using ADAP (Automated Data Analysis Pipeline) deconvolution in MZmine 2.53 under default parameters [37], comprising peak detection, baseline correction, and deconvolution [38]. Prior to multivariate analysis, the feature intensity table was normalized by sum and auto-scaled to reduce variance bias across metabolites. Relative abundances of identified compounds were also calculated as the mean ± standard deviation of peak areas for each treatment. In addition, the quantification of female pheromone components in gland extract was performed by constructing calibration curves based on peak areas from six serial dilutions (0.64 to 2000 ng/mL) of commercial standards: $y = 5.7 \times 10^{11}x + 9.3 \times 10^8$ for Z9-14:Ac, and $y = 5.6 \times 10^{11}x + 7.3 \times 10^9$ for Z9-14:OH under the same analytical conditions. An internal standard (*n*-decyl acetate, 4 ng/mL; Sigma-Aldrich, Milwaukee, MI, USA) was added to monitor instrumental performance and ensure consistency. Results were expressed as nanograms of standard per gland (ng/gland), and relative response factors were applied to adjust peak areas. All quantitative analyses were conducted in triplicate. Method precision was validated through intra- and inter-day analyses of standards, yielding relative standard deviations (RSD%) of 1.5–1.7% and 2.1–2.4%, respectively. The method's limit of detection (LOD) and limit of quantification (LOQ) were determined to be 10.6–12.6 pg/mL and 22.4–24.5 pg/mL, respectively.

2.4. Statistical Analysis

Post hoc statistical power analyses were performed for the major pheromone comparisons to assess the sensitivity of the pairwise tests. For each pairwise contrast, we calculated Cohen's *d* from group means and pooled standard deviations and used a two-sample *t*-test power function ($\alpha = 0.05$) to estimate achieved power. Additionally, the filtered and pre-processed FIT was further analyzed using the MetaboAnalyst 5.0 web platform [39] for comprehensive multivariate interpretation. Heatmap visualizations were generated to illustrate the overall distribution patterns of the extracted gland components across samples. To evaluate chemical differences associated with the age and diet, sparse partial least squares discriminant analysis (sPLS-DA) and ANOVA–Simultaneous Component Analysis (ASCA) were performed. Additionally, Multivariate Empirical Bayes Analysis (MEBA) was employed to investigate dynamic variation patterns in the chemical profiles as a function of age and diet. Finally, for the variables longevity, fecundity, and duration of phenological stages, analysis of variance (ANOVA) for a completely randomized design was used. The survival data were subjected to angular transformation to obtain an approximation to the normal distribution. The assumptions of normality and homogeneity of variances were validated using the Shapiro–Wilk and Bartlett tests.

3. Results and Discussion

3.1. Effect of Diet Type on Reproductive Parameters of *C. decolora*

The life cycle of *C. decolora* showed no statistical differences between diet treatments ($p > 0.05$). The total average duration of individuals fed on *Alstroemeria*, cauliflower, and the artificial diet was 80 ± 7.5 , 77.0 ± 7.2 , and 77.8 ± 14 days, respectively. The sex ratio across all diets was 1:1 (female–male). In addition, no statistical differences were observed ($p > 0.05$) in the duration of each biological stage. However, the embryonic development period (egg stage) was the shortest, lasting seven days for all diets. The larval stage exhibited the most extended duration, with averages of 33.9 ± 6.74 days on cauliflower, 35.14 ± 11.2 days on *Alstroemeria*, and 35.28 ± 13.7 days on the artificial diet. Adults that had been fed with *Alstroemeria* during their larval stage exhibited the highest mean longevity (21.3 ± 7.3 days), followed by those fed with cauliflower (19.0 ± 5.6 days) and the artificial diet (18.3 ± 12.3 days). These findings are generally consistent with previous reports, although specific comparisons reveal both similarities and slight variations in developmental parameters. For instance, the total duration of the life cycle observed in this study (ca. 80 days across the three tested diets) closely matches the lifespan reported by Vázquez-Covarrubias et al. [11] for *C. decolora* reared on an artificial diet [12] under controlled conditions (20 ± 5 °C and $50 \pm 5\%$ RH), which was approximately 76 days. However, some differences can be observed, which may be attributed not only to dietary composition but also to variations in experimental conditions, particularly temperature variations, known to influence developmental rates in *C. decolora* species [5]. Further comparisons with *C. incommoda* studies provide additional context. Flores-Pérez et al. [40] reported a total life-cycle duration of 75 days for *C. incommoda* reared on cauliflower, which is slightly shorter than the results observed for *C. decolora*. Similarly to the present study, larval duration represented the longest developmental stage, highlighting the critical role of this stage in determining overall life-cycle length. Moreover, the consistent 1:1 sex ratio observed across different diets and species emphasizes the stability of this parameter within the genus *Copitarsia*.

In contrast, the survival of the *C. decolora* population revealed distinct trends based on diet treatment. Survival was approximately 80% during the first 10 days for all diets. Beyond this period, survival on cauliflower decreased rapidly to 60% around day 20 and remained constant until day 55. Contrarily, survival on *Alstroemeria* declined gradually to 40% by day 60, followed by a sharp decrease between days 76 and 80. Survival on the artificial diet remained constant at 80% until day 60, after which it gradually declined. Individuals fed on cauliflower survived until approximately day 80, while those on the artificial diet survived up to day 100. The survival rate of the insects fed *Alstroemeria* dropped sharply after day 76.

Populations fed with cauliflower and artificial diets were characterized by high mortality during early stages, followed by a period of low and consistent mortality, and a subsequent sharp increase. In contrast, *Alstroemeria*-fed populations exhibited a relatively constant mortality rate across all stages of development. These differences may reflect variations in nutrient availability, secondary metabolite content, or other factors affecting larval growth and survival. Previous studies support these observations. For instance, Acatitla-Trejo et al. [27] found lower survival rates in *C. incommoda* larvae fed artificial diets based on cauliflower compared to those based on wheat or corn, likely due to nutritional deficiencies [41]. In general, *C. decolora* and related *Copitarsia* species feeding on *Alstroemeria* leaves in Colombia exhibited higher survival rates and faster development than individuals reared on less preferred diets or under less favorable environmental conditions [10,42].

In addition, the fertility of *C. decolora* females also demonstrated an apparent effect because of the diet. Females fed with *Alstroemeria* during the larval stage exhibited their peak fertility between days 7 and 10 (maximum on day 8, 312 ± 23 eggs per female). Those fed with cauliflower peaked between days 9 and 13 (maximum on day 11, 74 ± 11 eggs per female). Finally, females reared on the artificial diet displayed their peak fertility between days 15 and 17, with a maximum on day 16 (71 ± 16 eggs per female). Females from the *Alstroemeria* diet had an average cumulative oviposition of 1361 ± 115 eggs per female. Qualitatively, larvae reared on the artificial diet exhibited a unique developmental issue during the pupal stage. A lack of tegument between the pterotheca (part of the pupa case that covers the rudimentary wings) and the anterior abdominal segments was observed, leading to exposed tissue and subsequent wing atrophy in the emerged adults. Furthermore, fertility revealed a significant diet effect ($p > 0.05$) on the number of eggs laid per female. Larvae fed on *Alstroemeria*, the primary host plant, exhibited higher fertility rates compared to those fed on cauliflower or artificial diets. This pattern mirrors findings for *C. incommoda* reported by Flores-Pérez et al. [40], where larvae fed on host plants showed peak oviposition between 2 and 8 days, with a maximum on day 4. These results highlight the influence of diet on reproductive success, as the nutritional quality of larval diets directly impacts adult fitness and fecundity. The morphological and chemical characteristics of the diet, including the content of phytochemicals, likely contribute to these differences. Awmack & Leather [43] demonstrated that nutritional quality and metabolite presence affect insect development, survival, and reproduction, particularly during critical stages like larval and pupal development.

3.2. Female Pheromone Gland Components

GC–MS analysis revealed a complex chemical profile of the pheromone glands of *Copitarsia decolora* virgin females, comprising 26 compounds distributed across several chemical classes, including fatty acids and derivatives, alkanols and derivatives, and linear and branched alkanes (Table 1). Among these, alkanes predominated, accounting for approximately 61% of the gland's total chemical composition, while alkanols and their derivatives represented 35%, and fatty acids and related compounds constituted the remainder. All interpretations concerning pheromone chemistry are expressed in terms of relative abundance percentages, serving exclusively to depict compositional proportions among detected compounds.

Two main types of alkanes were detected, i.e., linear (35%) and branched (26%), with the latter primarily consisting of methyl-substituted derivatives. The dominance of alkanes, particularly heptacosane, hexacosane, and tetracosane, underscores their structural and functional role in maintaining the cuticular matrix and potentially modulating pheromone release rates [44]. These high-molecular-weight hydrocarbons are commonly associated with cuticular protection and species-specific recognition in Lepidoptera, suggesting a dual role in both physiological maintenance and communication [44–46].

Alkanols and alkyl acetates comprised another major chemical class, ranging from C14 to C20. They included functional groups such as hydroxyl (–OH), formyl (–CHO), and ester (–COOR) moieties, which were primarily located at the terminal (C1) position. This chemical diversity highlights the biosynthetic flexibility of *C. decolora* females, particularly their ability to produce oxygenated derivatives from fatty acid precursors characteristic of type-I pheromone systems in Noctuidae [47]. Among these compounds, Z9-14:OH and Z9-14:Ac have been previously identified as the main components of the sex pheromone emitted by *C. decolora* females [12]. Such metabolic flexibility may confer several adaptive advantages to this polyphagous pest [48]. For instance, it allows females to fine-tune pheromone composition in response to environmental or physiological conditions, thereby optimizing mate attraction and contributing to ecological success. Our

findings showed that Z9-14:Ac was reliably detected at relatively high levels, consistently exceeding 1.5% relative abundance across all diet treatments and age groups from 1 to 3 days (Table 1). Its highest relative abundance occurred in *Alstroemeria*-fed females, rising from $4.97 \pm 0.22\%$ at age 1 (E1) to $23.32 \pm 1.33\%$ at age 3 (E3). This sharp increase suggests an age-dependent proportional accumulation of the main pheromone component, regarding the other gland compounds, possibly linked to the maturation of the pheromone gland and the onset of sexual receptivity [49]. Similar but less pronounced patterns were observed for tetradec-1-yl acetate (14:Ac), which also peaked in *Alstroemeria*-fed females ($3.71 \pm 0.11\%$ at E3). In contrast, Z9-14:OH was absent in artificial-diet extracts. It was detected only at low levels (0.64%) in cauliflower-fed females at E3, suggesting that diet composition can drive compositional shifts among compounds within the same sample group. The predominance of acetates over alcohols (Table 1), along with the chain length (C10–C18), aligns with typical type-I sex pheromone compositions observed across lepidoptera and noctuid species [50,51]. Such pheromones play a fundamental role in long-range mate attraction and species recognition, supporting previous reports of Z9-14:Ac as a key sex attractant in *C. decolora* [12,13].

Table 1. Composition of the sex pheromone gland of *Copitarsia decolora* fed with three diets at three female adult ages.

Compound ^a	Rt ^b	RI ^c	RI ^d	Alstroemeria			Artificial			Cauliflower		
				Relative Abundance (%) ^e								
				E1 ^f	E2 ^f	E3 ^f	E1 ^f	E2 ^f	E3 ^f	E1 ^f	E2 ^f	E3 ^f
1 tetradecane	14.2	1400	1400	0.86 ± 0.19	0.97 ± 0.21	0.68 ± 0.10	1.52 ± 0.30	1.66 ± 0.22	2.56 ± 0.57	1.47 ± 0.28	1.92 ± 0.31	1.21 ± 0.22
2 4,11-dimethyltetradecane	16.3	1491	1483	1.17 ± 0.19	0.87 ± 0.15	0.59 ± 0.02	2.04 ± 0.44	2.13 ± 0.27	2.91 ± 0.20	1.32 ± 0.31	2.48 ± 0.36	1.92 ± 0.24
3 2,6,19-trimehyltetradecane	17.6	1547	1548	0.68 ± 0.19	0.61 ± 0.12	0.52 ± 0.02	1.11 ± 0.18	1.14 ± 0.13	1.27 ± 0.22	0.89 ± 0.14	1.07 ± 0.18	1.00 ± 0.11
4 hexadecane	18.9	1600	1600	1.91 ± 0.51	1.96 ± 0.28	1.27 ± 0.33	2.94 ± 0.37	2.42 ± 0.22	3.63 ± 0.38	2.17 ± 0.35	3.02 ± 0.43	2.45 ± 0.16
5 tetradec-9-en-1-ol	20.4	1667	1664	0.76 ± 0.12	0.51 ± 0.14	0.94 ± 0.22	nd	nd	nd	nd	nd	0.64 ± 0.11
6 heptadecane	21.2	1700	1700	1.01 ± 0.05	0.94 ± 0.21	0.65 ± 0.11	2.13 ± 0.53	1.99 ± 0.34	2.05 ± 0.16	1.71 ± 0.33	2.74 ± 0.55	1.87 ± 0.22
7 2,6,11,15-tetramethylhexadecane	22.2	1746	1753	1.41 ± 0.18	1.23 ± 0.31	1.13 ± 0.31	2.11 ± 0.34	1.82 ± 0.41	2.29 ± 0.55	1.24 ± 0.08	2.52 ± 0.52	2.04 ± 0.17
8 tetradec-9-en-1-yl acetate	23.2	1795	1793	4.97 ± 0.22	6.74 ± 1.25	23.32 ± 1.33	1.55 ± 0.14	2.11 ± 0.19	8.41 ± 1.08	2.79 ± 0.55	2.67 ± 0.29	6.86 ± 0.98
9 octadecane	23.4	1800	1800	3.15 ± 0.80	3.13 ± 0.33	2.02 ± 0.23	3.76 ± 0.92	3.42 ± 0.35	4.26 ± 0.66	2.61 ± 0.25	3.68 ± 0.97	3.54 ± 0.27
10 tetradec-1-yl acetate	23.5	1805	1793	1.01 ± 0.18	1.38 ± 0.16	3.71 ± 0.11	nd	nd	1.83 ± 0.22	0.64 ± 0.12	0.79 ± 0.14	1.61 ± 0.20
11 nonadecane	25.7	1900	1900	1.26 ± 0.28	0.97 ± 0.26	0.72 ± 0.09	1.11 ± 0.18	1.69 ± 0.34	1.45 ± 0.27	1.49 ± 0.22	2.57 ± 0.66	1.85 ± 0.29
12 2-methyl nonadecane	26.5	1950	1557	1.02 ± 0.21	1.17 ± 0.04	0.73 ± 0.11	1.23 ± 0.23	1.28 ± 0.22	1.33 ± 0.26	nd	1.61 ± 0.18	1.66 ± 0.33
13 <i>n</i> -hexadecanoic acid	26.6	1956	1942	1.32 ± 0.24	0.34 ± 0.01	1.85 ± 0.34	0.70 ± 0.17	1.26 ± 0.34	0.37 ± 0.03	nd	1.87 ± 0.23	0.63 ± 0.04
14 eicosane	27.4	2000	2000	4.11 ± 1.14	4.01 ± 0.43	2.37 ± 0.23	4.55 ± 1.08	5.04 ± 0.63	4.35 ± 0.94	2.04 ± 0.35	4.85 ± 1.13	4.12 ± 0.29
15 octadeca-1-ol	29.0	2086	2070	2.56 ± 0.55	1.61 ± 0.09	0.84 ± 0.12	1.99 ± 0.52	2.87 ± 0.29	1.39 ± 0.22	3.41 ± 0.62	2.42 ± 0.20	1.47 ± 0.23
16 octadec-9,12,15-trienal	30.4	2023	2010	3.26 ± 0.31	5.5 ± 0.82	2.91 ± 0.36	4.82 ± 1.22	4.86 ± 0.85	1.81 ± 0.11	0.49 ± 0.05	0.53 ± 0.02	0.34 ± 0.08
17 2,4-dimethyl eicosane	31.1	2095	2085	6.35 ± 0.14	nd	2.62 ± 0.44	0.97 ± 0.15	nd	6.26 ± 0.53	4.56 ± 0.32	5.18 ± 1.12	3.84 ± 0.39
18 octadec-9-enal	32.5	2137	2129	6.83 ± 1.42	4.97 ± 0.96	1.39 ± 0.15	1.88 ± 0.24	1.99 ± 0.25	1.78 ± 0.21	1.82 ± 0.28	3.20 ± 0.45	1.77 ± 0.28
19 eicos-9-enal	32.7	2184	2190	1.70 ± 0.15	2.85 ± 0.43	1.41 ± 0.14	1.21 ± 0.24	1.95 ± 0.34	1.55 ± 0.13	1.63 ± 0.24	1.77 ± 0.17	2.25 ± 0.26
20 2,4,10-trimethyl eicosane	32.9	2189	2185	2.55 ± 0.31	2.16 ± 0.34	3.15 ± 0.16	3.33 ± 0.11	1.89 ± 0.33	3.33 ± 0.52	0.83 ± 0.08	2.01 ± 0.01	1.25 ± 0.10
21 eicos-9-en-1-ol	33.9	2213	2220	1.21 ± 0.34	0.87 ± 0.19	2.12 ± 0.36	1.79 ± 0.09	1.56 ± 0.25	1.65 ± 0.30	0.68 ± 0.17	0.74 ± 0.21	0.87 ± 0.12
22 tetracosane	35.2	2400	2400	5.13 ± 0.63	6.76 ± 0.72	2.62 ± 0.41	5.79 ± 1.07	7.55 ± 1.51	6.26 ± 1.11	4.65 ± 0.11	6.04 ± 0.31	4.65 ± 1.19
23 unknown	37.5	2458	2442	4.67 ± 1.01	4.51 ± 1.01	2.29 ± 0.28	3.04 ± 0.72	3.36 ± 0.44	2.16 ± 0.42	2.33 ± 0.54	4.55 ± 1.03	0.89 ± 0.06
24 pentacosane	39.4	2500	2500	5.61 ± 1.11	5.38 ± 1.32	4.44 ± 1.18	6.24 ± 1.08	7.20 ± 0.94	6.67 ± 0.48	4.50 ± 0.19	6.49 ± 1.15	4.41 ± 1.13
25 hexacosane	41.2	2600	2600	8.08 ± 1.39	7.84 ± 0.91	5.17 ± 0.73	5.51 ± 1.17	7.77 ± 1.64	5.20 ± 0.23	4.61 ± 0.77	7.52 ± 1.47	5.51 ± 0.52
26 heptacosane	43.2	2700	2700	14.02 ± 2.52	12.54 ± 0.31	18.72 ± 2.16	6.66 ± 1.11	15.35 ± 1.85	11.57 ± 1.78	4.22 ± 0.91	12.58 ± 1.59	10.48 ± 1.51

^a Identified compound based on the mass spectra and Retention Index analyses; ^b Retention time (minutes) (Rt); ^c Experimental Retention Index; ^d Reported Retention Index; ^e Relative abundance according to the peak in the gas chromatogram after auto scaling; ^f Three female age groups were explored (1–2 (E1), 3–4 (E2), and 5–6 (E3) days post-emergence). nd = not detected.

Compounds containing 18-carbon chains, such as octadec-9-en-1-ol, octadec-9,12,15-trienal, and octadec-9-enal, showed a progressive decline in relative abundance with adult age across all diets. This pattern may reflect oxidative degradation or metabolic channeling of long-chain fatty aldehydes toward acetate synthesis as the females mature.

Conversely, 20-carbon-chain compounds (e.g., eicos-9-enal and eicos-9-en-1-ol) displayed 1–4% relative abundances and tended to increase with age, suggesting a proportional modulation during the later stages of glandular maturation.

The chemical composition of the pheromone glands was clearly influenced by both diet and age. Females reared on *Alstroemeria* consistently exhibited higher concentrations of Z9-14:Ac and a more complex oxygenated compound profile than those fed on cauliflower or artificial diets. The Z9-14:Ac/Z9-14:OH ratio, reaching approximately 25:1 in *Alstroemeria*-fed females, mirrors ratios previously associated with optimal male attraction in field assays [6,12,13], reinforcing the ecological relevance of diet in pheromone relative composition. In contrast, females from artificial diets produced markedly lower semi-quantitative levels of pheromone-related compounds, despite the diet's suitability for larval development and survival. This observation suggests a nutritional trade-off, where cost-effective rearing diets may sustain growth but fail to preserve key compositional traits, e.g., those components required for sexual signaling. This case supports condition-dependent sexual signaling, linking pheromone composition to fitness variation and heritable differences in mate attraction efficiency [52].

Age-dependent changes were also consistent and biologically meaningful. The accumulation of C14-based acetates with advancing age in virgin females, coupled with the decline of C18-based aldehydes and alcohols, suggests an intrinsic shift in relative composition [53]. Such proportional adjustments likely reflect age-related modulation of enzymatic activities (e.g., acetyltransferase or desaturase), mediated by the pheromone biosynthesis-activating neuropeptide (PBAN), which regulates the relative balance among pheromone components rather than their total output [54,55]. Broadly, Table 1 highlights how dietary inputs and physiological age jointly influence the chemical composition of the pheromone gland in *C. decolora*. These findings emphasize the metabolic flexibility of this species and provide valuable insights for refining semiochemical-based pest management strategies.

Taken together, these compositional patterns reveal that both larval diet and adult age exert significant influences on the chemical profile of the female pheromone glands. Although individual compound analyses reveal specific age- or diet-dependent variations in relative composition, a broader understanding of how these factors interact to define the overall glandular chemical phenotype requires an integrative, multivariate approach. Accordingly, multivariate analyses of the pheromone gland profiles were performed to examine global clustering patterns, identify discriminant compounds, and evaluate how nutritional and physiological variables jointly modulate the semiochemical composition of *C. decolora*.

3.3. Multivariate Analysis of the Chemical Profiles of *C. decolora* Female Pheromone Glands

Two-factor principal component analysis (PCA) was applied to explore global patterns in the FIT-based chemical composition of *C. decolora* female pheromone glands, considering both larval diet and adult age (Figure 1). The 3D PCA score plot (Figure 1a) revealed a clear multivariate separation among treatments (85.9% of the total variance), with the first two components (PC1 and PC2) explaining most of the variance (59.3% and 17.9%, respectively). A clear clustering pattern was observed, with females reared on *Alstroemeria* (Als) at older ages (E3) forming a distinct group (green-shaded ellipsoid), indicating that both larval diet and adult age markedly influence the relative chemical composition of the pheromone gland. Individuals fed on cauliflower (Cau) also formed a separate cluster along PC1, clearly distinguished from those reared on Als and the artificial diet (Art), underscoring the strong effect of the larval nutritional source on the adult chemical phenotype. Within each dietary group, additional clustering by age was evident, reflecting compositional adjustments associated with physiological maturation. The tendency of older

females (E3), particularly those fed on Als, to cluster toward positive values of PC1 and PC2 values suggests cumulative, age-related shifts in proportional compound representation within the gland.

The PCA loading plot (Figure 1b) identified the compounds contributing most to these compositional differences. Pheromone-related compounds, such as Z9-14:Ac and 14:OAc, were the primary discriminants along PC1, being strongly associated with *Alstroemeria*-fed females and emphasizing their importance in defining diet-dependent chemical signature. These acetates are well-known semiochemical constituents or biosynthetic derivatives in noctuid pheromone systems, highlighting how host-plant-derived nutrition can influence the relative allocation of compounds within the pheromone blend [30]. Other compounds, including long-chain hydrocarbons and aldehydes, contributed to variation along PC2 and PC3, representing secondary metabolic differences related to both age and diet.

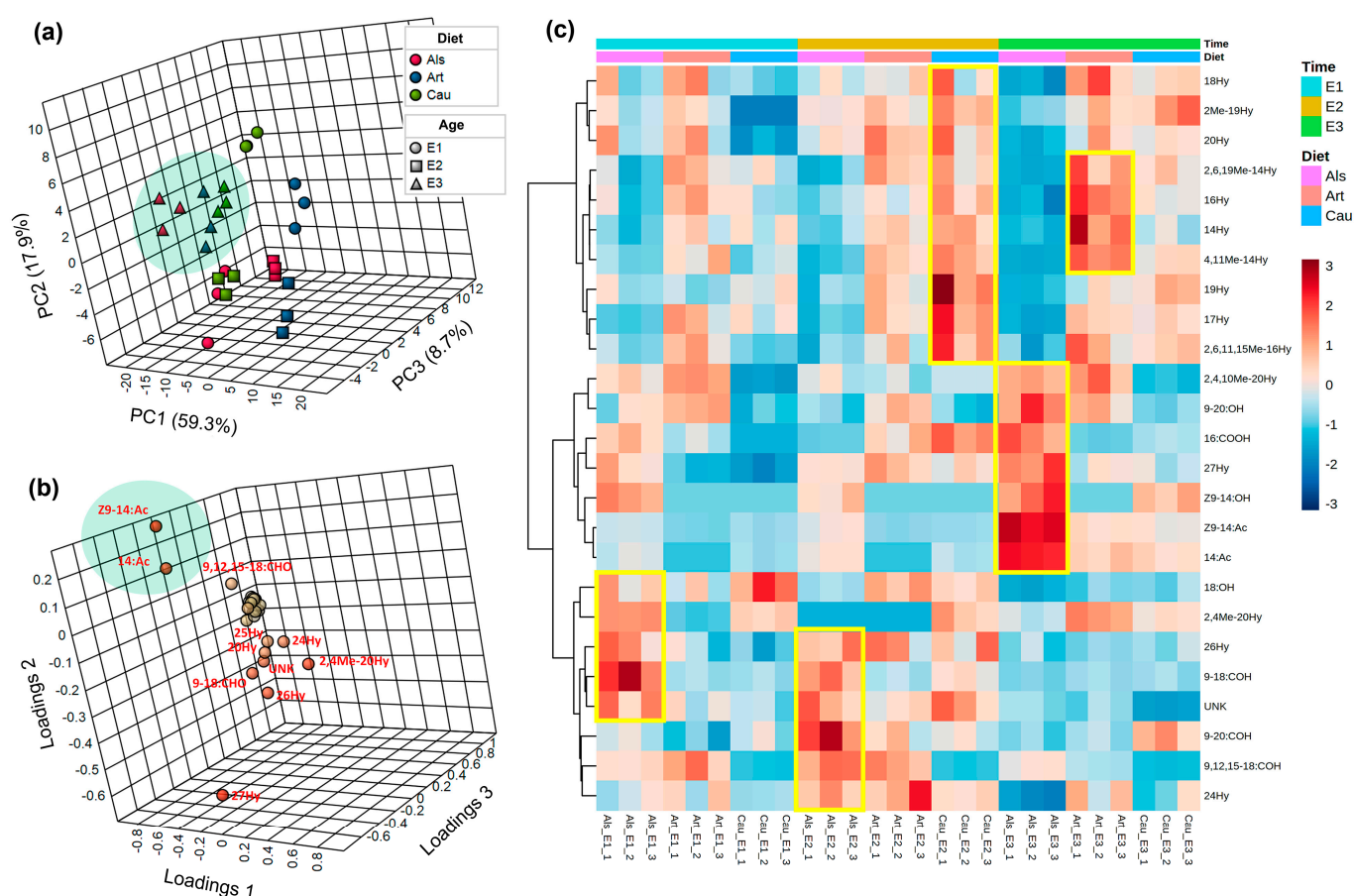


Figure 1. Two-factor principal component analyses (PCA) and heatmap-based distribution of female pheromone gland chemical profiles of *C. decolora* reared on three diets (*Alstroemeria* (Als), artificial (Art), and cauliflower (Cau)) and sampled at three female adult age periods (E1: 1–2 days, E2: 3–4 days, E3: 5–6 days). Compounds are abbreviated according to the identification listed in Table 1. (a) Two-factor, three-dimensional (3D) PCA score plot of individual gland extracts showing separation based on diet and age. (b) Three-dimensional PCA loading plot, indicating the contribution of individual chemical compounds to sample separation; pheromone-related compounds are highlighted in a green ellipsoid. (c) Heatmap with hierarchical clustering of compounds (rows) and samples (columns), visualizing normalized feature-based abundance across treatments. The diet and age combinations are indicated by colored bars at the top. Red cells indicate higher compound abundance, as indicated by the color scale (red: high, blue: low) in the heatmap. Yellow boxes highlight regions where specific compound clusters appeared to be more abundant.

The heatmap visualization (Figure 1c) complements the PCA by depicting normalized feature-based compound abundances across treatments. Distinct metabolic clusters were consistently evident, with the yellow-framed regions highlighting groups of compounds that were dependably more abundant in females fed specific diets and at certain ages. Notably, Als-fed females displayed a higher abundance of pheromone-related compounds, identified in the loading plot, during the later adult stage (E3), which displayed a higher relative abundance of pheromone-related compounds [54]. In contrast, alkanals and long-chain *n*-alkanes exhibited differential abundance patterns at earlier stages (E1 and E2). Alkanes were particularly enriched in females reared on the Cau and the Art diets at E2 and E3, respectively, indicating that diet composition influences the proportional accumulation of hydrocarbon-based compounds associated with cuticular or gland functions. Females reared on the Art diet showed comparatively lower and more uniform signal intensities, indicating a restricted compound profile. Conversely, those reared on cauliflower exhibited intermediate patterns, suggesting that nutritional input may partially sustain compositional balance, possibly by reduction in lipid droplets in the pheromone glands [31]. These multivariate patterns demonstrated that the chemical phenotype of the female pheromone glands in *C. decolora* is dynamically shaped by nutritional and physiological factors, resulting in diet- and age-dependent shifts in gland composition.

The multivariate analysis was further expanded towards diet supervision. Hence, the sPLS-DA scores plot (Figure 2a) revealed a more precise separation among the chemical profiles of female pheromone glands according to the larval diet, with three well-defined clusters corresponding to individuals reared on the three test diets. The first component (43.8% of explained variance) accounts for significant discrimination among groups, while the second component (12.4%) contributes to intra-group variation. This pattern corroborates that the chemical composition of the pheromone gland is particularly diet-dependent.

The larval nutrition trend is distinctly illustrated by the ASCA model, which further supports these findings by partitioning the contributions of diet and age effects on gland chemistry. The scores for the main effect of diet (Figure 2b) show contrasting trajectories across the three age stages (E1–E3). The Cau group maintains consistently higher positive scores, while the Als and Art profiles shift markedly as females age. This trend suggests that females derived from cauliflower-fed larvae retain a distinct chemical profile throughout adult maturation. On the other hand, the diet $\times$ age interaction (Figure 2c) underscores the dynamic nature of gland composition over time. While Als-reared females show a decrease in scores with age, Cau-derived individuals exhibit an opposite trend, increasing markedly at later stages (E3). The Art group remains intermediate but relatively stable, indicating a less pronounced modulation of chemical components. These contrasting trajectories imply that both developmental and nutritional factors jointly modulate the relative composition of pheromone gland metabolites, possibly affecting pheromone emission patterns and mating communication efficiency.

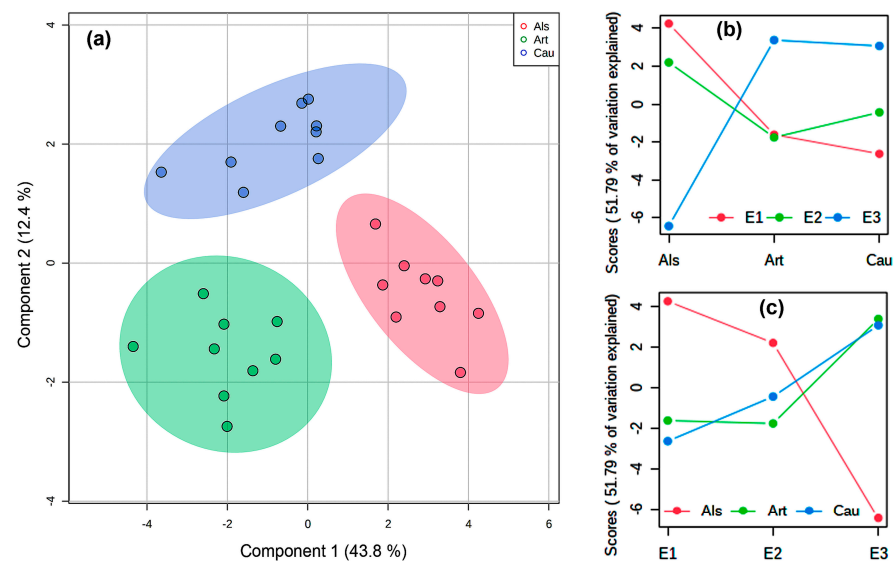


Figure 2. Discrimination of female pheromone gland chemical profiles in *C. decolora* by diet (*Alstroemeria*: Als, Artificial: Art, Cauliflower: Cau) and female adult age periods (E1: 1–2 days, E2: 3–4 days, E3: 5–6 days) using sparse partial least squares discriminant analysis (sPLS-DA) and ANOVA–Simultaneous Component Analysis (ASCA) models. (a) sPLS-DA scores plot showing distinct clustering based on diet: Als, Art, and Cau. (b) ASCA scores for the main effect of diet across three female age stages (E1: 1–2 days, E2: 3–4 days, E3: 5–6 days). (c) ASCA scores for the interaction between diet and age, highlighting divergent trends in gland composition over time. Together, the results demonstrate strong diet- and age-dependent variation in pheromone gland chemical makeup, with implications for pheromone production and pest management.

The MEBA time-series analysis (Figure 3) provides a detailed view of how diet and adult age jointly shape the temporal dynamics of major pheromone gland metabolites in *C. decolora*. Across all compound classes, females reared on Als consistently exhibited higher and more age-dependent compound abundance, indicating that larval host plants sustain pheromone composition. In particular, the acetate esters Z9-14:OAc and 14:OAc exhibited marked accumulation in Als-fed females at the oldest age stage (E3), although this pattern deviated somewhat from the typical timing of pheromone release associated with peak reproductive activity [56]. These patterns were strongly discriminant, as indicated by Hotelling's T^2 values (773–825), confirming a robust diet-dependent divergence in titer capacity. Such increases likely reflect enhanced acetylation of alkanol precursors under optimal nutritional conditions provided by the *Alstroemeria* host [30]. Conversely, oxygenated derivatives such as 18:OH and 9-18:OH showed decreasing trends with age in Als-fed females but remained relatively stable or increased in those reared on artificial or cauliflower diets. This observation suggests that the conversion of long-chain alcohols into their corresponding acetates may occur more efficiently in females reared on their natural host, thereby reducing the pool of intermediate alcohols at later ages, possibly as a result of acetylation involving nonspecific substrates [57]. However, Z9-14:OH, the other identified pheromone component, exhibited slightly higher levels at stages E1 and E3, although it can be considered to maintain a constant titer across ages. The polyunsaturated aldehyde 9,12,15-18:CHO displayed transient peaks at E2 in Als-fed females, consistent with a potential role as a biosynthetic intermediate or an oxidative by-product of fatty acid metabolism preceding pheromone emission. Notably, females from the cauliflower diet maintained lower and less dynamic levels of this compound, suggesting a restricted chemical composition [31]. Females reared on suboptimal diets (Art or Cau) displayed blunted or asynchronous temporal patterns, possibly leading to reduced mating signal efficacy. In contrast to most lepidopteran species, where pheromone titers typically

decline with age [58], this study demonstrates that older virgin females of *C. decolora* exhibit significantly higher pheromone levels in their pheromone glands than younger individuals.

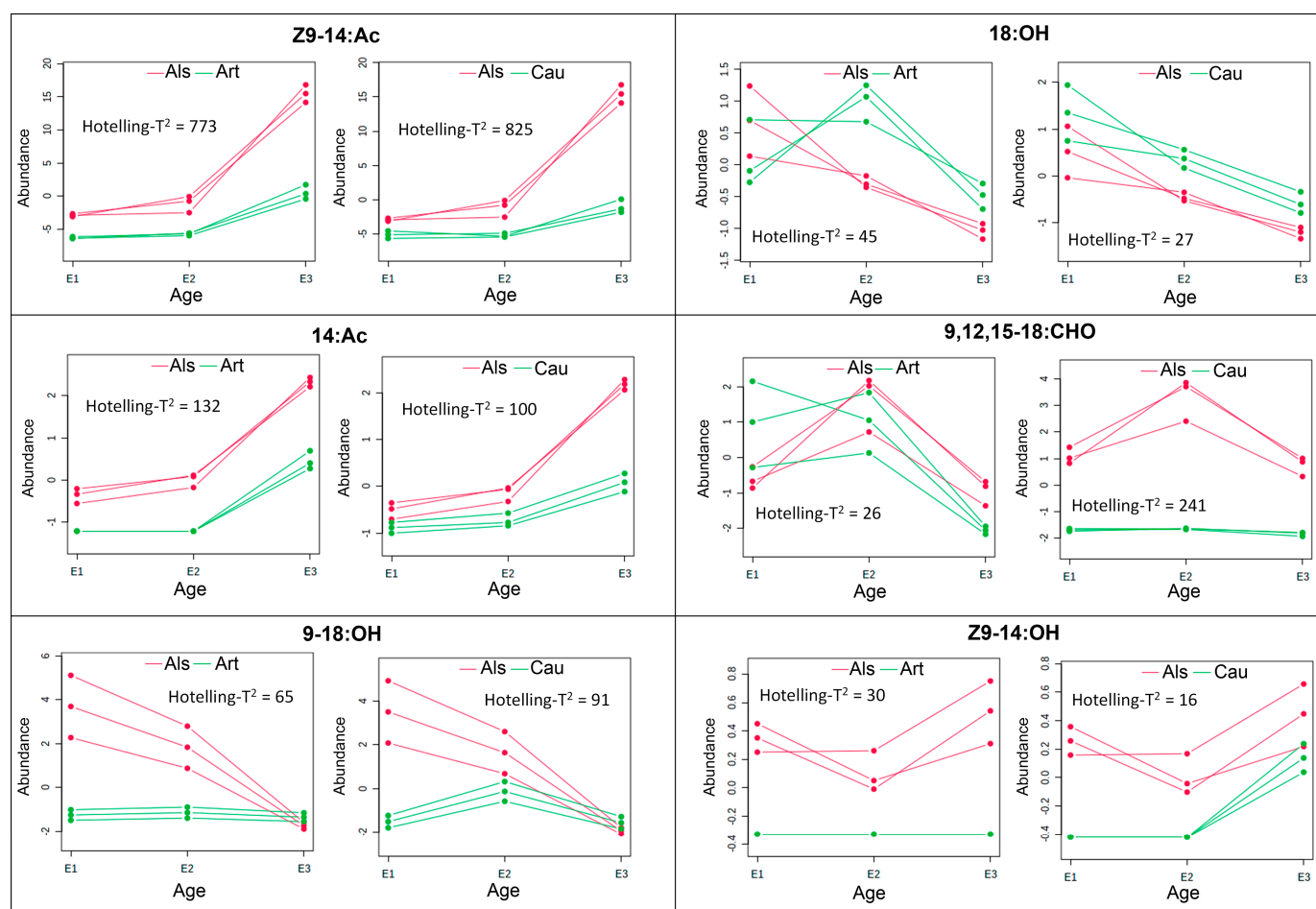


Figure 3. Temporal dynamics of key pheromone gland compounds in *C. decolora* females according to diet (Alstroemeria: Als, Artificial: Art, Cauliflower: Cau) and female adult age periods (E1: 1–2 days, E2: 3–4 days, E3: 5–6 days), modeled by Multivariate Empirical Bayes Analysis (MEBA). Each panel includes Hotelling's T^2 values, reflecting the statistical separation between diet groups.

Quantitative analysis of the two primary pheromone components, Z9-14:Ac and Z9-14:OH, demonstrated the influence of both larval diet and adult age on pheromone titer in *C. decolora* female gland (Table 2). The acetate (Z9-14:Ac) was consistently the dominant compound across all treatments, while the corresponding alcohol (Z9-14:OH) occurred at lower concentrations and was frequently undetectable in females reared on artificial and cauliflower diets at early stages (E1–E2).

Table 2. Concentrations (ng/gland) of sex pheromone components of *Copitarsia decolora* fed with three diets at three ages.

Diet	Alstroemeria			Artificial			Cauliflower		
Age	E1 ^b	E2 ^b	E3 ^b	E1 ^b	E2 ^b	E3 ^b	E1 ^b	E2 ^b	E3 ^b
Z9-14:OH ^a	0.012 ± 0.002	0.004 ± 0.0005	0.038 ± 0.005	nd	nd	nd	nd	nd	0.013 ± 0.001
Z9-14:Ac ^a	1.05 ± 0.21	2.45 ± 0.19	9.52 ± 1.23	0.095 ± 0.012	0.37 ± 0.02	0.95 ± 0.03	0.85 ± 0.03	2.88 ± 0.16	1.96 ± 0.26

^a Primary components of the *C. decolora* sex pheromone: Z9-14:Ac = (Z)-tetradec-9-enyl acetate and Z9-14:OH = (Z)-tetradec-9-enol. ^b Three female age stages: E1 = 1–2 days, E2 = 3–4 days, E3 = 5–6 days). nd = not detected.

In females reared on Als, Z9-14:Ac levels increased markedly with age, rising almost tenfold from E1 (1.05 ± 0.21 ng/gland) to E3 (9.52 ± 1.23 ng/gland). A similar, though less pronounced, trend was observed in females from the cauliflower diet, which reached moderate Z9-14:Ac levels at E2 (2.88 ± 0.16 ng/gland) but showed a slight decline at E3. In contrast, females reared on the artificial diet consistently produced lower levels of Z9-14:Ac throughout adult life, indicating that nutritional limitation during larval development can suppress the biosynthetic capacity of the pheromone gland. The alcohol precursor Z9-14:OH was detected exclusively in Als- and Cau-fed females, with notably higher levels in the Als group at the latest age stage (E3 = 0.038 ± 0.005 ng/gland). Its absence in females from the artificial diet and early developmental stages may reflect restricted fatty acyl precursor pools or limited enzymatic reduction capacity under suboptimal larval nutrition. Alternatively, the absence of Z9-14:OH in females reared on the artificial diet may reflect limited availability of Δ^9 -desaturated C14 fatty acid precursors (e.g., myristoleic acid), which serve as substrates for pheromone alcohol biosynthesis [31]. Such lipid deficiencies are common in meridic or oligidic formulations lacking specific unsaturated fatty acids [26]. This attenuated pheromone production observed in artificial- and cauliflower-fed females may contribute to reduced attractiveness, providing insights into the nutritional and ecological regulation of chemical communication in this pest species.

3.4. Implications for Rearing and Pest Management

The larval diet plays a critical role in shaping adult physiological and reproductive traits in *C. decolora*, particularly those associated with sex pheromone biosynthesis [30]. Our results indicated that the nutritional quality directly influenced the chemical composition and abundance of *C. decolora* pheromone gland components, demonstrating that diet-mediated metabolic plasticity extends to signaling systems. This finding has broad implications for both laboratory rearing protocols and pest management strategies targeting *C. decolora*.

From a rearing perspective, artificial diets offer clear operational advantages, such as reduced contamination risk, batch consistency, and ease of handling, but may inadvertently compromise adult performance [59]. The present results indicate that females reared on artificial media exhibited markedly reduced levels of pheromone components (Z9-14:Ac and Z9-14:OH) and lower overall chemical diversity in the pheromone gland. Such reductions may correlate with decreased mating success or altered behavioral responses, potentially encouraging the exploration of optimal and beneficial artificial diets in large-scale rearing programs intended for biological control, behavioral assays, or sterile insect technique (SIT) applications [60]. Optimization of artificial diet formulations to better mimic the nutrient balance of natural hosts (e.g., *Alstroemeria* foliage) could therefore improve the physiological and reproductive quality of laboratory populations.

In an applied context, the detailed characterization of pheromone composition and its modulation by diet and age provides valuable insights for refining pheromone-based pest management strategies. The observed ratios and concentrations of the known pheromone components, i.e., Z9-14:Ac and Z9-14:OH, serve as a biochemical reference for developing modified pheromone blends to be employed in Colombian Andean environments and specific crops. This information is also relevant for determining the optimal timing for field trap placement. Our study suggests that peak pheromone production in *Alstroemeria* occurs at 5–6 days of age in females. In this case, pheromone traps should be placed in the field just before the first cohort of wild females in the population reaches this peak calling age. This ensures that the traps are fully active and competitive when the first peak of male moth activity is expected. These formulations can enhance field monitoring systems, enabling early detection of adult populations and more effective timing of control interventions [61]. Previous field trials with related *Copitarsia* species have

demonstrated the efficacy of binary pheromone mixtures in attracting males to traps deployed in cauliflower crops, underscoring the potential for translational application of these findings [6].

Beyond immediate pest-control applications, this study lays a foundation for exploring how environmental and nutritional factors influence chemical communication in noctuid moths. Integrating these chemical ecology insights into rearing and field management could facilitate the design of more sustainable, species-specific control strategies [62]. Future work should assess how variations in pheromone signal strength and composition under different larval diets affect male behavioral responses in semi-field and field conditions. Such studies will clarify whether dietary optimization can enhance the efficacy of pheromone-based monitoring or mating disruption programs and contribute to long-term, ecologically grounded pest management frameworks for *C. decolora* and related species.

4. Conclusions

This study demonstrates that both larval diet and adult age markedly influence the chemical composition of female pheromone glands in *C. decolora*. Multivariate and quantitative analyses revealed strong diet-dependent plasticity in pheromone biosynthesis, with *Alstroemeria*-fed females exhibiting higher abundance of the key components Z9-14:Ac and Z9-14:OH, particularly at later adult stages. These findings highlight the metabolic adaptability of *C. decolora* and its capacity to modulate pheromone production in response to nutritional and physiological factors. Such chemical flexibility likely contributes to the species' reproductive success and ecological persistence across diverse cropping systems. From an application perspective, the defined pheromone ratios and their diet-age modulation provide a valuable reference for improving synthetic lures and pheromone-based monitoring tools. Generally, this work advances understanding of biochemical determinants of pest communication, offering novel insights for developing nutritionally informed and semiochemically guided crop protection strategies.

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