Graphical Abstract

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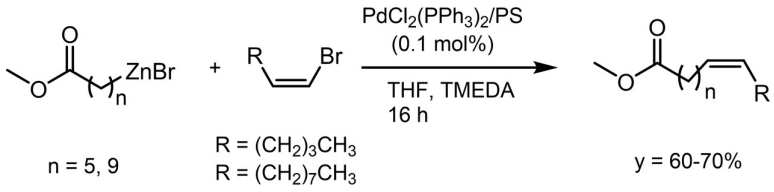

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Identification and synthesis of new sex-specific components of olive fruit fly (*Bactrocera oleae*) female rectal gland, through original Negishi reactions on supported catalysts

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Olive Fruit Fly
Negishi coupling
Organozinc compounds
Supported catalysis
Pheromones synthesis

ABSTRACT

In the present study, eleven new sex-specific components extracted from female rectal gland of olive fruit flies were synthesized and identified. The quantitative determination of those components by GC and GC/EI-MS, at different moments of the insect life span, highlighted the growing trend of their secretion. While for the synthesis of saturated esters, conventional transesterification methods could be adopted, for the synthesis of unsaturated components, a Negishi cross-coupling between organozinc halides and (*Z*)-1-bromo-alkenes was developed. To the extent of our knowledge, this reaction represents the first example of catalyst-supported Negishi coupling, between an alkyl electron donor and an alkenyl electron acceptor.

1. Introduction

1.1. State of the war against olive fruit fly (Bactrocera oleae)

Since Plato's sacred olive tree stood up over the most famous academic institution, the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is known as the major pest of olive crops worldwide.[1] This invasive pest, widely known in the Mediterranean region, threated California olive crops as well.[2] The substantial economic impact of the damage losses of fruits and oil quality is dealt adopting different strategies, like insecticide-based control (e.g., dimethoate and fenthion),[3] biological control using natural enemies,[4] sterile insect techniques,[5] attraction to baits[6] lured with colors[7] and pheromones.[8] A pesticide-free approach would be of course the most appealing, for many reasons, including the risk for human and environmental health, not to mention the insurgence of pesticide resistance.[9,10] In our group, in the past years, we focused mainly on the development of semiochemicals-based control approaches. This standpoint requires a deep knowledge of the exact chemical components of sex-specific pheromones, at different life age and conditions of the insects. The story of the discovery of olive fruit fly sex-specific pheromones is complex and sometimes controversial. It is well-known that *B. oleae* females attract conspecific males by using racemic 1,7-dioxaspiro[5,5]undecane (olean),[11] which was used for monitoring the insect with different strategies (lure and kill or pheromone-based mating disruption), leading to fluctuating results.[12] The failure of those preliminary tests on the guided control against *B. oleae*, could be attributed to the lack of knowledge on its intraspecific chemical communication. In our efforts to solve this issue, in the past decade, we identified (*Z*)-9-

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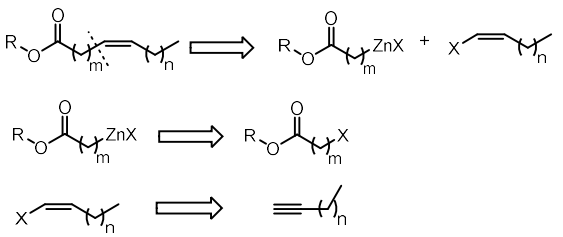
tricosene (muscalure) as male-produced female attractant,[13,14] and ten carboxilic esters as female-produced components, two of which with intraspecific semiochemical activity.[15] However, from GC/MS chromatograms of female rectal gland extracted, eleven components were not unequivocally identified and quantified, because relating standards were not commercially available. From the fragmentation analysis of those components, we speculated the presence of four saturated isobutyl esters (decanoate, dodecanoate, tetradecanoate, hexadecanoate), two hexadecenoate methyl esters, two hexadecenoate ethyl esters, two octadecenoate methyl esters and one octadecenoate ethyl ester. To confirm our hypothesis, we synthesized the corresponding molecules, developing as well, a new methodology for supported-catalyst Negishi coupling, between an alkyl electron donor and an alkenyl electron acceptor.

1.2. Choice of synthetic strategies

For the synthesis of saturated esters, we chose a convenient esterification and transesterification method proposed by Kaimal et al.,[16] using catalytic amount of molecular iodine and an excess of the desired alcohol. The method is simple, mild and non-toxic, generally leading to high yields, regardless of potentially sensitive functional groups present in the molecule. For the synthesis of monounsaturated esters, we focused on synthetic strategies which lead mainly to the formation of (*Z*)-olefines, as in nature, fatty acids are mainly present in *cis* conformation, with some rare exceptions.[17] Furthermore, it is essential to obtain high stereoisomeric purities, because even small amount of the undesired isomers could bias the bioactivity results of pheromonic components. Several methods, for the synthesis of (*Z*)-monounsaturated esters are available in literature, starting from the most classic Wittig olefination. For example, Murata et al. in the efforts to obtain [C6-$^2H_2$]-dioleoylphosphatidylcholine, synthesised ethyl (*Z*)-tridec-4-enoate, through a Wittig reaction with 50% yield.[18] Many other authors described similar results with a major formation of the *Z*-alkene. Notably, Darwish reported the preparation of conformationally pure deuterated (*Z*)-methyl oleate via the Wittig reaction, with 60% yield.[19] Another strategy is the well-known and generally efficient, stereoselective catalytic hydrogenation of the corresponding alkyne. The alkyne, however, must be previously synthesised, through the formation of Lithium acetylides, like shown by Miyoshi in the synthesis of some cardiolipins,[20] or by Kronenberg for the synthesis of natural *Sphingomonas* glycolipids.[21] Such step is not compatible with the presence of ester functions, so in our case, we would have needed some extra-steps for the protection of the functional group. We evaluated more appealing, for our purpose, an approach based on cross-coupling reactions catalysed by transition metals, especially for the possibility of improving convenience and greenness of the method, using heterogeneous catalysis. It is indeed largely recognized that supported catalysts, even though generally requiring harsher conditions, can be re-used, presenting as well, the advantage to avoid expensive purifications. Nevertheless, for the synthesis of biologically interesting molecules, supported-catalysts cross-coupling reactions, are still limited. There are several interesting examples of C_{sp3}-C_{sp2} Suzuki couplings reported in literature, for the selective synthesis of stereo chemically pure (*Z*) ω-alkenoates.[22] The same Suzuki showed the alkylation reaction through boronic esters on bromo-acrylate and 3-bromo-5,5-dimethylcyclohex-2-en-1-one, with 60-70% yields.[23] Using an excess of Ag_2O, the group of Falck improved the reaction between boronic acids and alkyl and aryl electrophiles additive.[24] Furthermore, adding triphenylarsine, Bellina et al. could synthesise unsymmetrically disubstituted 3,4-dialkyl-2(5H)-furanones.[25] Interestingly, Santelli et al. exploiting a less air-sensitive bidentate complex PdCl(dppb)(C_3H_5) as pre-catalyst, and Cs_2CO_3 as base, could achieve C_{sp3}-C_{sp2} *coupling*, with low catalyst loading (1-2 mol%) and without the use of expensive Ag_2O.[26] However, alkyl boronic acids are difficult to handle, as a result of their instability in the air. Moreover, catalyst loadings are generally too high (1-10 mol%) and there are still no examples in literature of Suzuki cross-coupling reactions between alkenyl halides and organo-boron species, catalysed by supported catalyst. We estimated more convenient an approach based on Negishi cross-coupling reactions, between organo-zinc compounds and alkenyl halides,[27] like described in the retrosynthetic scheme reported on Scheme 1.



Scheme 1. Retrosynthetic approach of the Negishi cross-coupling reaction

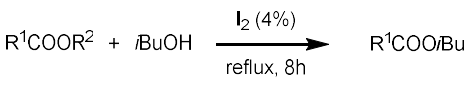
Even though the preparation of organozinc halides through classic metal-metal exchange is not compatible with the presence of ester functions, it is possible to use a direct oxidative addition of Zn (0). Knochel et al.,[28] for example, prepared organozinc halides from alkyl-iodides, activating Zinc powder with 1,2-dibromoethane and trichloromethylsilane.[29] Alternately, on alkyl-bromide, Hou et al. used moleculare iodine and *N,N*-dimethylacetamide.[30] Knochel and other authors added LiCl salt, to remove from the metal surface, the already formed organozinc compound.[31,32,33] Generally, Negishi reactions can be performed easily with low-cost catalysts, like $Pd(PPh_3)_4$ or $PdCl_2(PPh_3)_2$ in THF, and as showed by Tamaru et al., it is possible to synthesise stereo specifically, both *cis* and *trans* olefins, with high purity and good yields.[34] Due to its efficiency, Negishi coupling was used also in the synthesis of rather complex molecules, like (-)-rasfonin,[35] and mycestericin A.[36] Most relevant, for our needs, was the study of Lipshutz et al. on the stereo chemistry of the cross coupling with (Z) alkenyl halides,[37] which strongly depends on the catalyst ligand. It was indeed reported that excellent results, in terms of selectivity and yields, are reached using $PdCl_2(PPh_3)_2$/TMEDA, with only 1 mol% catalyst loading. Even though organozinc halides are air-sensitive, Negishi reaction, at least for alkyl-alkenyl coupling, looks to have better yields with lower catalyst loading, respect to Suzuki reaction. Until now, also in the case of Negishi coupling, there are no example of supported catalysis, between an alkyl electron donor and an alkenyl electron acceptor and this contribution represent a first example.

2. Results and Discussion

2.1. Synthesis of saturated isobutyl esters

The isobutyl transesterification reactions were performed through the already described molecular iodine method,[16] using commercially available starting materials and obtaining good yields, as shown in Table 1. To exclude the possibility of confusing the isomeric nature of butyl esters, *n*-butyl, *sec*-butyl and *tert*-butyl esters were synthesized as well. Once confirmed, by their different retention time on GC analysis, that only the *iso*-butyl ester isomers were present in the female rectal gland extracted, only those were completely isolated and characterized.

Table 1. Synthesis of saturated *iso*-butyl esters.



$R^1 = C_9H_{19}$-, $C_{11}H_{23}$-, $C_{13}H_{27}$-, $C_{15}H_{31}$-
$R^2 = CH_3CH_2$- or CH_3-

R^1	R^2	Product	GC Conv.[a]	Yield[b]
C_9H_{19}-	CH_3CH_2-	*i*Bu decanoate (**1**)	90%	88%
$C_{11}H_{23}$-	CH_3CH_2-	*i*Bu dodecanoate (**2**)	90%	88%
$C_{13}H_{27}$-	CH_3CH_2-	*i*Bu tetradecanoate (**3**)	90%	85%
$C_{15}H_{31}$-	CH_3-	*i*Bu hexadecanoate (**4**)	90%	87%

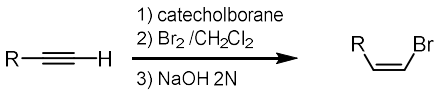
[a] Conversion evaluated as ratio between the product area and the s.m. area
[b] Isolated yield by flash chromatography on silica gel

2.2. Synthesis of alkenyl esters

2.2.1 Preparation of (Z)-alkenyl halides

The intermediates (*Z*)-alkenyl halides, which have to be used in the following Negishi coupling, were prepared through well-known synthetic procedures. The approach is based on the initial regio and stereo controlled *syn*-hydroboration of the corresponding alkyne with catecholborane. Bromine is then added *onepot* on the obtained boronic ester, to give addition to the double bond with an *anti*-mechanism. Finally, the alkenyl bromine is obtained by *anti*-elimination reaction with sodium-hydroxide as a base. This method allowed us to synthesize (*Z*)-1-bromohex-1-ene (**5**), (*Z*)-1-bromodec-1-ene (**6**) and (*Z*)-1-bromooct-1-ene (**12**) with good yields, depending on the purification method used, as shown in Table 2. Crucial for our synthesis was the high stereoisomeric purity of the compounds, that once obtained were directly used for the following steps, without further characterizations.

Table 2. Synthesis of alkenyl halides



$R = C_4H_9$-, C_8H_{17}-

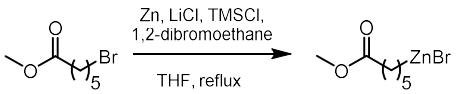
Alkyne	Product	*Z/E* ratio[c]	Yields
1-hexyne	(Z)-1-bromohex-1-ene (**5**)	99:1	51%[a]
1-decyne	(Z)-1-bromodec-1-ene (**6**)	99:1	85%[b]

[a] Product purified by low pressure fractional distillation
[b] Product purified by flash chromatography

2.2.2. Preparation of organozinc halides

As mentioned in section 1.2, transmetalation is not a convenient method for our organozinc intermediates, due to the high reactivity of ester functional groups to organometallic species. A suitable approach is the direct oxidative addition of Zn (0), which has been described and improved by Knochel in 2006,[31] adding 1 equivalent of LiCl and 2 equivalents of Zinc powder, previously activated with 10 mol% of 1,2-dibromoethane and 1 mol% of Trimethylsilyl chloride (TMSCl). We used the Knochel method on (*Z*)-1-bromohex-1-ene (**5**), testing different conditions, to obtain 5-methoxycarbonyl-1-pentylzinc bromide (**7**), as shown in Table 3. The use of LiCl was indeed necessary for the reaction, which otherwise would not happen. Anyway, considering the long reaction time of 44 h, to obtain a complete conversion, we increased the amount of 1,2-dibromoethane up to 20 mol% and TMSCl up to 5 mol%, added in two portions as described in the experimental section, shortening the reaction time to 24 h. Noteworthy, in all the 3 conditions tested in Table 3, we observed traces of methyl 6-chlorohexanoate as byproduct.

Table 3. Optimization of organozinc halides formation



Entry	1,2-dibromethane (mol %)	TMSCl (mol %)	LiCl (equiv.)	Time (h)	GC Conv.[a]
1	10	1	0	29	0%
2	10	1	1	20, 29,44	45%,82%, 100%
3	20	5	1	24	97%

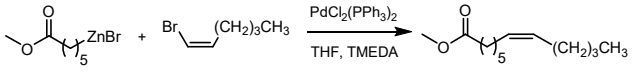
[a] Conversion evaluated as ratio between the product area and the s.m. area

With the optimized conditions, (10-methoxy-10-oxodecyl) zinc(II) bromide (**8**) and (8-methoxy-8-oxooctyl)zinc(II) bromide (**13**) were also synthesized with similar results, as described in the experimental section. The THF solutions of **7** and **8** were titrated by iodometry, using a literature method,[38] as described in the experimental part, generally resulting with a title comprehended between 0.56 M and 0.73 M.

2.2.3. Negishi Cross-Coupling reaction on homogeneous catalysis

Coupling reaction was, in the first place, tested for the synthesis of Methyl (*Z*)-dodec-7-enoate (**9**), on homogeneous catalysis, using $PdCl_2(PPh_3)_2$/TMEDA, as this couple was reported in literature to give excellent selectivity and yields. The results were indeed satisfactory, as shown in Table 4, with high yield, short reaction time and preserving the *Z/E* ratio (99:1). In the attempt to decrease the catalyst loading, we found 0.05 mol% of $PdCl_2(PPh_3)_2$, to be a good compromise to obtain a good yield in 2 hours reaction time.

Table 4. Optimization of Negishi coupling

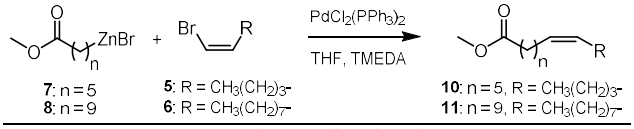


Entry[a]	$PdCl_2(PPh_3)_2$ (mol%)	Time (h)	GC Conv.[b]	GC Yield[c]	Yield[d]
1	1	2	100%	-	83%
2	0.5	2	100%	79%	-
3	0.1	2	100%	78%	-
4	0.05	2	100%	76%	71%
5	0.025	20	79%	-	-
6	0.01	20	25%	-	-

[a] Reaction conditions: 1.2 equiv. of **7**, THF (3.1 mL/mmol of **5**), 1.2 equiv. of TMEDA, reflux, argon atmosphere
[b] Conversion evaluated as ratio between the product area and the s.m. area
[c] GC yield calculated using naphthalene as internal standard
[d] Product purified by flash chromatography

Using this optimized condition, also Methyl (*Z*)-hexadec-7-enoate (**10**) and Methyl (*Z*)-hexadec-11-enoate (**11**) were synthesized in good yields and complete stereoselectivity, as shown in Table 5.

Table 5. Synthesis of alkenyl esters



Entry[a]	Product	Organozinc halide	Alkenyl halide	Time (h)	GC Conv.[b]	Yield[c]
1	**10**	**7**	**6**	2	100%	78%
2	**11**	**8**	**5**	2	100%	69%

[a] Reaction conditions: 0.05 mol% of $PdCl_2(PPh_3)_2$, 1.2 equiv. of **7** or **8**, THF (3.1 mL/mmol of **5** or **6**), 1.2 equiv. of TMEDA, reflux, argon

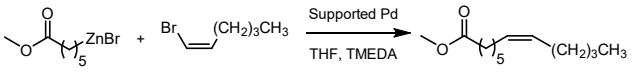
[b] Conversion evaluated as ratio between the product area and the s.m. area

[c] Product purified by flash chromatography

2.2.4. Negishi Cross-Coupling reaction on supported catalysts

As highlighted in the introduction, despite the several advantages of supported-catalysts, their use for the synthesis of biologically interesting molecules is still limited, and there are currently no examples of Negishi supported catalysis between an alkyl electron donor and an alkenyl electron acceptor. Therefore, we tested three different commercially available supported catalysts, mainly $PdCl_2(PPh_3)_2$/PS, an analogue of $PdCl_2(PPh_3)_2$ in which Pd (II) is complexed through phosphines in a cross-linked system, FibreCat™ 1007, in which the phosphonic groups coordinating Pd(II) are attached to the end tails of polymeric fibers, and PdEncat™, where Pd(II) is locked inside a polyurea matrix. Optimal conditions were investigated again on the synthesis of **9**, with the results reported in Table 6. Stereoselectivity was still retained with a *Z/E* ratio of 99:1, with all the three catalysts, even though, generally, longer reaction times were required to reach completion. However, with $PdCl_2(PPh_3)_2$/PS and FibreCat™ 1007, we were able to decrease the catalyst loading down to 0.1 mol%, with good yields, in 14 hours reaction time, which is fairly acceptable for supported catalysts.

Table 6. Optimization of Negishi cross-coupling on Supported catalysts



En-try.[a]	Catalyst	Pd (mol%)	Time (h)	GC Conv.[b]	GC Yield[c]	Yield[d]
1	$PdCl_2(PPh_3)_2$/ PS	2	2	100%	83%	-
2	FibreCat™ 1007	2	2	100%	80%	-
3	$PdCl_2(PPh_3)_2$/ PS	0.5	2 14	57% 100%	81%	-
4	$PdCl_2(PPh_3)_2$/ PS	0.1	2 14	22% 100%	76%	67%
5	FibreCat™ 1007	0.1	14	100%	76%	68%
6	PdEncat™	0.1	26	10%	-	-

[a] Reaction conditions: 1.2 equiv. of **7**, THF (3.1 mL/mmol of **5**), 1.2 equiv. of TMEDA, reflux, argon atmosphere

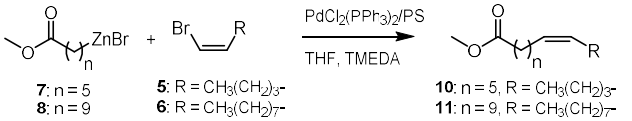
[b] Conversion evaluated as ratio between the product area and the s.m. area

[c] GC yield calculated using naphthalene as internal standard

[d] Product purified by flash chromatography

With our best conditions, we synthesized **10** and **11** as well, using the cheapest $PdCl_2(PPh_3)_2$/PS, as shown in Table 7, with consistent results.

Table 7. Synthesis of alkenyl esters on supported catalyst



Entry[a]	Product	Pd (mol%)	Time (h)	GC Conv.[b]	Yield[c]
1	**10**	0.1	14	100%	73%
2	**11**	0.1	16	52%	-
3	**11**	0.3	14	100%	67%

[a] Reaction conditions: 1.2 equiv. of **7** or **8**, THF (3.1 mL/mmol of **5** or **6**, 1.2 equiv. of TMEDA, reflux, argon

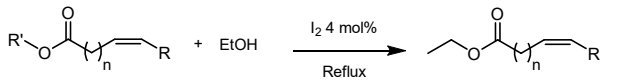
[b] Conversion evaluated as ratio between the product area and the s.m. area

[c] Product purified by flash chromatography

2.3 Transesterification of methyl alkenyl esters

To complete the list of compounds that we hypothesized, from the fragmentation analysis of female rectal gland extracted, in part *1.1.*, we synthesized the missing three ethyl alkenyl esters, with the same method described in part *2.1*, starting from commercially available Methyl (*Z*)-octadec-11-enoate (**12**), (*Z*)-hexadec-9-enoic acid (**13**), and the previously prepared Methyl (*Z*)-hexadec-7-enoate (**10**). The corresponding ethyl alkenyl esters, Ethyl (*Z*)-octadec-11-enoate (**14**), Ethyl (*Z*)-hexadec-7-enoate (**15**) and Ethyl (*Z*)-hexadec-9-enoate (**16**), were obtained with high yield and purity as shown in Table 8.

Table 8. Synthesis of ethyl alkenyl esters



Starting	Product	Time (h)	GC Conv.[a]	Yield[b]
12	Ethyl (Z)-octadec-11-enoate (**14**)	8	97%	91%
10	Ethyl (Z)-hexadec-7-enoate (**15**)	8	93%	86%
13	Ethyl (Z)-hexadec-9-enoate (**16**)	4	100%	87%

[a] Conversion evaluated as ratio between the product area and the s.m. area

[b] Product purified by flash chromatography

2.4. Identification and quantification of the unknown compounds in female B. oleae gland extracts.

Extracts from virgin female rectal glands after 1, 4, 9, 13, 17 and 21 days after the fly emergence, obtained as described in the experimental part *4.8.*, were analyzed by GC and GC/EI-MS, using for each age three different samples. To safely assign the right retention times and fragmentation patterns, we used two GC columns with different polar stationary phase. The exact superimposition of chromatograms and fragmentations allowed us to unambiguously confirm the presence in the rectal glands of **1**, **2**, **3**, **4**, **9**, **10**, **11**, **14**, **15**, **16** and the commercially available Methyl (*Z*)-octadec-9-enoate (**17**). None of these compounds were previously identified in female extracts, apart a generic *cis*-hexadecenoate. The eleven compounds were quantified in nanograms per gland (ng/fly), for each age investigated, through absolute GC calibration curves, as shown in Table 9. Means and standard deviations (SD) were calculated using absolute calibration curves, by injecting pure standards (each injection was replicated three times). Differences in the age-related production of chemicals

were analyzed using the generalized linear model described by Benelli et al.[39] with two factors, i.e., the fly's age (j=1-6) and identified chemical (j=1-10). Averages were separated by the Tukey's HSD test (P<0.05).

Table 9. Quantification in ng/fly of extract components at different ages

Comp.	1 day	4 days	9 days	13 days	17 days	21 days
1	0	0.1 (± 0.02)	1.2 (± 0.03)	5.7 (± 0.7)	7.2 (± 0.5)	11.8 (± 0.6)
2	0	1.4 (± 0.02)	10.4 (± 0.4)	29.6 (± 1.2)	40.8 (± 1.6)	79.6 (± 1.4)
3	0	4.1 (± 0.1)	30.1 (± 2.3)	86.1 (± 3.6)	111 (± 3)	186 (± 3)
4	0	2.8 (± 0.3)	33.6 (± 2.7)	83.3 (± 1.7)	121 (± 3)	176 (± 4)
10	0	0.4 (± 0.04)	4.6 (± 0.08)	5.0 (± 0.5)	4.1 (± 0.2)	8.4 (± 0.4)
11	0	0.05 (± 0.01)	0.2 (± 0.02)	1.0 (± 0.05)	1.0 (± 0.1)	2.4 (± 0.1)
12	0	0.6 (± 0.03)	4.5 (± 0.4)	16.6 (± 0.8)	24.1 (± 2.4)	9.3 (± 0.5)
14	0	2.1 (± 0.06)	3.2 (± 0.09)	12.3 (± 1.1)	16.7 (± 0.7)	32.7 (± 2.4)
15	0	2.7 (± 0.2)	4.2 (± 0.07)	5.8 (± 0.4)	4.9 (± 0.3)	31 (± 1.8)
16	0	4.0 (± 0.1)	4.0 (± 0.1)	16.3 (± 1.0)	12.8 (± 0.6)	43.4 (± 1.6)
17	0	0.7 (± 0.03)	7.8 (± 0.3)	12.9 (± 1.9)	19.0 (± 0.8)	41.7 (± 2.2)

As previously observed for *olean* in the case of females, and *muscalure* for males, the amount of the identified esters increases with the age, until a maximum of secretion after 21 days from fly emergence. The linear trend estimation of the new identified sex-specific components is clearer in Figure 1. After 21 days, the production range of the esters varied from a minimum of 2.4 ng/fly for **11** to a maximum of 186 ng/fly for **3**. These values are low compared to the production of *olean* for female flies, that in the same condition fluctuate between 3000 and 4000 ng/fly, but it's proportionate to *muscalure* production in males (50 ng/fly after 21 days).

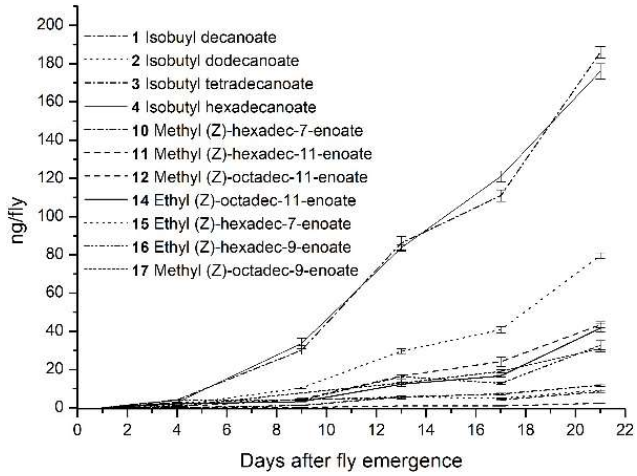


Figure 1. Productions of identified components as a function of age after fly emergence

Noteworthy, the amounts of compounds **3** and **4** look to exponentially increase after the 4th day. Considering that sexual maturation arises after 8 days for female flies, these compounds could play an important role in sexual communication between the insects. Anyway, this hypothesis will be evaluated only in future, through behavioral and electrophysiological experiments.

3. Conclusion

This work represents a significant step forward into the guided control of olive fruit fly, through the identification of 11 new sex-specific components in female rectal glands. To effectively develop, from these data, a semiochemical-based olive-fly fight, further analyses are needed, especially to confirm the reproducibility in different years and in different periods of the year. Moreover, for the synthesis of alkenyl esters, a supported-catalyst Negishi coupling, between an alkyl electron donor and an alkenyl electron acceptor, has been developed, which is a novelty in the field of supported *cross-coupling*.

4. Experimental section

4.1 General Information

All reactions where performed under Argon atmosphere, handling solvents and reagents using hypodermic syringes conveniently stored in a desiccator. Dichloromethane was dried over calcium hydride and distilled while Tetrahydrofuran was dried over potassium and distilled. When specified, solvents where purged through Argon bubbling. Catalysts $PdCl_2$(dppf), $PdCl_2(PPh_3)_2$, $PdCl_2(PPh_3)_2$/PS (Pd: 1.00 mmol/g), FibreCatTM 1007 (Pd: 0.72 mmol/g), PdEnCatTM 40 (Pd: 0.42 mmol/g) where purchased by Sigma-Aldrich. Zinc Powder had a particle-size distribution < 10 μm (Sigma-Aldrich, lot. SHBF7346V). Reactions were followed by GC and GC/EI-MS on small reaction samples (30-100 μL), treated with the same work-up described in general procedures. Purifications of the reaction crude were performed by flash chromatography using Silica Gel 60 (40-63 μm, Sigma Aldrich), or by fractional distillation. TLC silica gel plates were purchased by Merck (Merck 60 F254). Gas chromatography (GC) analyses were performed on a Dani GC 1000 with PTV injector, FID detector and two bonded FSOT columns (Alltech, 30 m×0.25 mm i.d., 0.25 μm): AT-5 (column 1) and AT-35 (column 2). Gas chromatography–mass spectrometry (GC/MS) analyses were performed with an Agilent apparatus: mass selective detector 5973 Network, 6890 N Network GC system and HP-5MS bonded column (30 m×0.25 mm i.d., 0.25 μm, column 3). NMR spectra were recorded on Bruker 400 MHz, Bruker Avance II DRX 400 spectrometer and on Varian INOVA 600 MHz.

4.2. General Procedure (A) for esterification and transesterification with molecular iodine

In a typical experiment, in a 2-neck round-bottom flask, equipped with a reflux condenser, one equivalent of the corresponding acid or ester was dissolved in the opportune alcohol (0.60 mL/mmol), and 2 or 4% of Iodine were added. The solution was let stirring at reflux and monitored by GC until complete conversion. The crude solution was concentrated at reduced pressure and treated with a saturated solution of $Na_2S_2O_3$. The aqueous phase was then extracted three times with diethyl ether and the reunited organic fractions were washed with deionized water. The solution was dried over sodium sulfate, filtered and concentrated under reduced pressure. The obtained crude was then purified by flash chromatography.

*4.2.1 Isobuyl decanoate (**1**):*

Following general procedure (A), to a mixture of commercially available Ethyl Decanoate (1.93 mL, 8.3 mmol) and 5 mL of Isobuyl alcohol, 4 mol% of molecular Iodine I_2 (103.4 mg, 0.41 mmol) was added. The resulting solution was then stirred at reflux for 8 hours. The excess of alcohol was removed under reduced pressure and the residue was dissolved in diethyl ether. The

solution was washed with a solution of sodium thiosulfate and distilled water, dried over anhydrous sodium sulfate and concentrated in vacuo to yield the crude product, which was purified by flash chromatography on silica gel (Hex/Et_2O 90:10). The product was obtained as a colorless oil with 88% yield (purity 99% by GC). Spectroscopic data in agreement with literature.[40] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (d, *J* = 6 Hz, 2H), 2.31 (t, *J* = 8 Hz, 2H), 1.93 (m, 1H), 1.63 (m, 2H), 1.21-1.36 (bs, 12H), 0.93 (d, *J* = 8 Hz, 6H), 0.88 (t, *J* = 6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 174.1, 70.5, 34.5, 32.0, 29.5, 29.4, 27.9, 25.2, 22.8, 19.2, 14.2. IR (neat): 2958, 2924, 2857, 1740, 1466, 1393, 1376, 1348, 1245, 1164, 1110, 1060, 1004, 940, 912, 733 cm^{-1}; MS, m/z (%): 228(1), 173(67, 155(100), 143, (4), 129(20), 116(7), 97(5), 57(60), 41(30)

*4.2.2 Isobutyl dodecanoate (**2**):*

Following general procedure (A), to a mixture of commercially available Ethyl Laurate (1.10 mL, 4.15 mmol) and 2.5 mL of Isobuyl alcohol, 4 mol% of molecular Iodine I_2 (45 mg, 0.18 mmol) was added. The resulting solution was then stirred at reflux for 8 hours. The excess of alcohol was removed under reduced pressure and the residue was dissolved in diethyl ether. The solution was washed with a solution of sodium thiosulfate and distilled water, dried over anhydrous sodium sulfate and concentrated in vacuo to yield the crude product, which was purified by flash chromatography on silica gel (Hex/Et_2O 90:10). The product was obtained as a colorless oil with 88% yield (purity 99% by GC). Spectroscopic data in agreement with literature.[41] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (d, *J* = 8 Hz, 2H), 2.31 (t, *J* = 8 Hz, 2H), 1.93 (m, 1H), 1.63 (m, 2H), 1.37-1.21 (bs, 16H), 0.93 (d, *J* = 6 Hz, 6H), 0.88 (t, J = 6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 70.5, 34.5, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 27.9, 25.2, 22.8, 19.2, 14.2. IR (neat): 2952, 2924, 2852, 1737, 1466, 1418, 1379, 1281, 1256, 1234, 1172, 1110, 1013, 909, 803, 730, 722 cm^{-1}; MS, m/z (%): 256(2), 201(84), 183(100), 171 (6), 157(13), 129(16), 116(9), 57(65), 41(30).

*4.3.3 Isobutyl tetradecanoate (**3**):*

Following general procedure (A), to a mixture of commercially available Ethyl Myristate (2.47 mL, 8.28 mmol) and 5 mL of Isobuyl alcohol, 4 mol% of molecular Iodine I_2 (85 mg, 0.33 mmol) was added. The resulting solution was then stirred at reflux for 8 hours. The excess of alcohol was removed under reduced pressure and the residue was dissolved in diethyl ether. The solution was washed with a solution of sodium thiosulfate and distilled water, dried over anhydrous sodium sulfate and concentrated in vacuo to yield the crude product, which was purified by flash chromatography on silica gel (Hex/Et_2O 90:10). The product was obtained as a colorless oil with 85% yield (purity 98% by GC). Spectroscopic data in agreement with literature.[40] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (d, *J* = 7 Hz, 2H), 2.31 (t, *J* = 8 Hz, 2H), 1.93 (m, 1H), 1.63 (m, 2H), 1.36-1.22 (bs, 20H), 0.94 (d, *J* = 7 Hz, 6H), 0.89 (t, *J* = 7 Hz, 3H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 70.5, 34.5, 32.1, 29.81, 29.77, 29.60, 29.5, 29.4, 29.3, 27.9, 25.2, 22.8, 19.2, 14.2 IR (neat): 2958, 2924, 2852, 1737, 1465, 1396, 1376, 1376, 1348, 1242, 1169, 1113, 1012, 914, 732 cm^{-1}; MS, m/z (%): 284(4), 229(100), 211(98), 185(20),129(25), 116(12), 97(14), 57(91), 41(36).

*4.3.4 Isobutyl hexadecanoate (**4**):*

Following general procedure (A), to a mixture of commercially available Methyl Palmitate (844 mg, 3.12 mmol) and 1.88 mL of Isobuyl alcohol, 4 mol% of molecular Iodine I_2 (33 mg, 0.13 mmol) was added. The resulting solution was then stirred at reflux for 8 hours. The excess of alcohol was removed under reduced pressure and the residue was dissolved in diethyl ether. The solution was washed with a solution of sodium thiosulfate and distilled water, dried over anhydrous sodium sulfate and concentrated in vacuo to yield the crude product, which was purified by flash chromatography on silica gel (Hex/Et_2O 90:10). The product was obtained as a colorless oil with 87% yield (purity 99% by GC). Spectroscopic data in agreement with literature.[41] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (d, *J* = 7 Hz, 2H), 2.31 (t, *J* = 7 Hz, 2H), 1.93 (m, 1H), 1.63 (m, 2H), 1.34-1.22 (bs, 24H), 0.93 (d, *J* = 7Hz, 6H), 0.88 (t, *J* = 6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 174.1, 70.5, 34.5, 32.0, 29.81, 29.77, 29.71, 29.6, 29.5, 29.4, 29.3, 27.9, 25.2, 22.8, 19.2, 14.2. IR (neat): 2952, 2919, 2852, 1740, 1466, 1393, 1379, 1351, 1242, 1172, 1113, 1013, 733, 722 cm^{-1}; MS, m/z (%): 312(4), 257(77), 239(68), 213(11), 129(18), 116(12), 97(13), 56(100), 41(32).

*4.3.5 Ethyl (Z)-octadec-11-enoate **(14)**:*

Following general procedure (A), to a mixture of commercially available Methyl (Z)-octadec-11-enoate (**12**) (67.5 mg, 0.23 mmol) and 265 μL of Ethyl alcohol, 4 mol% of molecular Iodine I_2 (2.34 mg, 0.092 mmol) was added. The resulting solution was then stirred at reflux for 8 hours. The excess of alcohol was removed under reduced pressure and the residue was dissolved in diethyl ether. The solution was washed with a solution of sodium thiosulfate and distilled water, dried over anhydrous sodium sulfate and concentrated in vacuo to yield the crude product, which was purified by flash chromatography on silica gel (Hex/Et_2O 95:5). The product was obtained as a colorless oil with 91% yield (purity 99% by GC). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5,34 (tm, *J* = 5,5 Hz, 2H), 4,11 (q, *J* = 7,1 Hz, 2H), 2,27 (t, *J* = 7,6 Hz, 2H), 2,08 – 1,93 (m, 4H), 1,65-1,57 (m, 2H), 1,40–1,19 (m, 23H), 0,88 (t, *J* = 6,9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 130.0, 129.9, 60.3, 34.5, 31.9, 29.9, 29.6, 29.5, 29.4, 29.2, 29.1, 27.3, 25.1, 22.8, 14.4, 14.2. IR (neat): 2924, 2852, 1737, 1460, 1373, 1343, 1303, 1250, 1175, 1116, 1097, 1045, 722 cm^{-1}; MS, m/z (%): 310(8), 265(40), 222(27), 180(20), 123(22), 97(59), 88(63), 83(63), 69(79), 55(100), 41(47). Anal. Elem., Calc. per C20H38O2: C 77,36%; H 12,33%. Found: C 77,40%; H 12,38%

*4.3.6. Ethyl (Z)-hexadec-7-enoate (**15**):*

Following general procedure (A), to a mixture of synthesised Methyl (Z)-hexadec-7-enoate (**10**) (266 mg, 0.99 mmol) and 0.60 mL of Ethyl alcohol, 4 mol% of molecular Iodine I_2 (10 mg, 0.039 mmol) was added. The resulting solution was then stirred at reflux for 8 hours. The excess of alcohol was removed under reduced pressure and the residue was dissolved in diethyl ether. The solution was washed with a solution of sodium thiosulfate and distilled water, dried over anhydrous sodium sulfate and concentrated in vacuo to yield the crude product, which was purified by flash chromatography on silica gel (Hex/Et2O 95:5). The product was obtained as a colorless oil with 86% yield (purity 97% by GC). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5,34 (dt, *J* = 6,5 Hz, 2H), 4,12 (q, *J* = 7,1 Hz, 2H), 2,28 (t, *J* = 7,6 Hz, 2H), 2,04-1,94 (m, 4H), 1,66-1,58 (m, 2H), 1,39–1,20 (m, 19H), 0,87 (t, *J* = 7,0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 130.4, 129.6, 60.3, 34.5, 32.1, 29.9, 29.7, 29.5, 29.4, 28.9, 27.3, 27.2, 25.0, 22.8, 14.4, 14.3. IR (neat): 2997, 2924, 2857, 1737, 1460, 1371, 1348, 1301, 1250, 1178, 1116, 1032, 937, 859, 783, 722 cm^{-1}; MS, m/z (%): 282(18), 236(87), 194(63), 152(49), 101(75), 96(77), 88(84), 84(71), 69(66), 55(100), 41(63). Anal. Elem., Calc. per C18H34O2: C 77,54%; H 12,13%, Found C 74,57%; H 12,15%

*4.3.7 Ethyl (Z)-hexadec-9-enoate (**16**):*

Following general procedure (A), to a mixture of commercially available (Z)-hexadec-9-enoic acid (**13**) (100 mg, 0.39 mmol) and 0.25 mL of Ethyl alcohol, 4 mol% of molecular Iodine I_2 (4 mg, 0.016 mmol) was added. The resulting solution was then stirred at reflux for 4 hours. The excess of alcohol was removed under

reduced pressure and the residue was dissolved in diethyl ether. The solution was washed with a solution of sodium thiosulfate and distilled water, dried over anhydrous sodium sulfate and concentrated in vacuo to yield the crude product, which was purified by flash chromatography on silica gel (Hex/Et2O 95:5). The product was obtained as a colorless oil with 87% yield (purity 99% by GC). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5,38 (dt, *J* = 10,8 Hz, 2H), 4,12 (q, *J* = 7,1 Hz, 2H), 2,28 (t, *J* = 7,6 Hz, 2H), 2,04-1,94 (m, 4H), 1,66-1,58 (m, 2H), 1,39–1,20 (m, 19H), 0,87 (t, *J* = 6,9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 130.1, 129.9, 60.3, 34.5, 32.0, 29.9, 29.8, 29.3, 29.24, 29.22, 29.1, 27.34, 27.28, 22.8, 14.4, 14.3. IR (neat): 2924, 2852, 1737, 1463, 1371, 1348, 1301, 1245, 1178, 1115, 1094, 1035, 912, 853, 808, 727 cm^{-1}; MS, m/z (%): 282(14), 237(50), 219 (3), 194(43), 152(35), 101(61), 88(71), 69(81), 55(100), 41(58) Anal. Elem., Calc. per C18H34O2: C 77,54%; H 12,13%, Found C 74,59%; H 12,18%

*4.3. General procedure **(B)** for the synthesis of (Z)-1-bromo-1-alkenes*

In a typical experiment, in a 3-neck round-bottom flask, equipped with a dropping funnel and a reflux condenser, one equivalent of the corresponding alkyne and one equivalent of catecholborane were mixed, under argon. The mixture was stirred at 70°C for 3 hours, and afterwards cooled down at room temperature and diluted with dichloromethane (0.25mL/mmol). The solution was then cooled down at -10°C. A solution containing 2 equiv. of Bromine in dichloromethane (5 M) was added dropwise, over a period of one hour. The solution was then let stirring for an additional hour, at -10°C. The mixture was warmed up at 0°C, and 68 mL of NaOH 2N aqueous solution were added dropwise, over a period of 15 minutes. The reaction was let stirring for an additional hour at 0 °C. The obtained solution was extracted 3 times (3x 100 mL) with dichloromethane and the collected organic phases washed with brine until neutral pH. The solution was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography or distillation.

*4.3.1. (Z)-1-bromohex-1-ene (**5**):*

Following general procedure (B), 1.13 mL of 1-Hexyne (10 mmol), and 1 mL of catecholborane (10 mmol) were stirred, under argon, at 70 °C. Once cooled down, the mixture was diluted with 2.5 mL of dichloromethane and added dropwise with 4 mL of a dichloromethane solution containing Bromine (5 M). The reaction was quenched as described in the general procedure (B). The crude was purified by fractional distillation (226 mbar), obtaining the product as a colorless oil with a yield of 51% (purity 88% by GC, Z/E, 99:1). Once identified the product by NMR, the intermediate was used directly for the following steps. Spectroscopic data in agreement with literature.[42] ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.13 (d, J = 4 Hz), 6.10 (m, 1H), 2.21 (m, 2H), 1.33-1.44 (m, 4H), 0.93 (t, J = 4 Hz, 3H). MS, m/z (%): 164(43), 162(42), 121(18), 119(17), 83(50), 67(15), 56(100), 41(85). bp = 76-77 °C/226 mbar (lit. 96 °C/226 mbar)

*4.3.2. (Z)-1-bromodec-1-ene (**6**):*

Following general procedure (B), 1.8 mL of 1-Decyne (10 mmol), and 1 mL of catecholborane (10 mmol) were stirred, under argon, at 70 °C. Once cooled down, the mixture was diluted with 2.5 mL of dichloromethane and added dropwise with 4 mL of a dichloromethane solution containing Bromine (5 M). The reaction was quenched as described in the general procedure (B). The crude was purified by flash chromatography on silica gel, using hexane as a solvent, obtaining the product as a colorless oil with a yield of 85% (purity 98% by GC, Z/E, 99:1). Once identified the product by NMR, the intermediate was used directly for the following steps. Spectroscopic data in agreement with literature.[43] ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14 (d, J = 4 Hz, 1H), 6.09 (m, 1H), 2.20 (m, 2H), 1.42 (m, 2H), 1.30 (m, 10H), 0.89 (t, J = 4 Hz, 3H). MS, m/z (%): 220(9), 218(10), 150(11), 148(11), 121(16), 119(16), 97(48), 83(100), 69(51), 57(56), 41(51).

4.4. General procedure (C) for the preparation of organozinc halides through oxidative addition to alkyl bromides

In a 2-neck round-bottom flask equipped with a reflux condenser, under inert atmosphere, one equiv. of LiCl was added. The system was warmed up to 150-170°C, at reduced pressure (0.1 mbar), for 20 minutes. Two equiv. of Zinc powder were added, and the system was again warmed up to 150-170°C, at reduced pressure (0.1 mbar), for 20 minutes. The flask was let cooling down to room temperature and treated with 3 cycles of vacuum-argon. Afterwards dry THF (1mL/mmol of corresponding alkyl bromide) and 10 mol% of 1,2-dibromoethane were added. The mixture was let stirring at 65°C for 15 minutes and cooled down at room temperature. Depending on substrate, 1 or 5 mol% of Trimethylsilyl chloride were added, and the solution was warmed up to 65°C for 15 minutes and then cooled down to room temperature. The suitable alkyl bromide (1 equiv.) was then added dropwise and the solution stirred at reflux for 3 hours. The solution was cooled down to room temperature and 10 mol% of 1,2dibromoethane were added. The solution was again warmed up to reflux and the reaction was followed by GC, until complete conversion. Once the reaction went to completion, the flask was let cooling down at room temperature and Zinc was let precipitating over 6 hours. The titration of organozinc compound was carried out by iodometry, using a literature method.

4.5. General procedure (D) for Negishi coupling between organozinc halides and alkenyl halides

In a 2-neck round bottom flask, equipped with a reflux condenser and under inert atmosphere, was added the catalyst, in the form of $PdCl_2(PPh_3)_2$ or one of its supported version, $PdCl_2(PPh_3)_2$/PS, FibreCat 1007TM and PdEncat40TM, in the specific amounts indicated for each product synthesized. Taking into account, the volume of solvent already used to prepare the organozinc halide solution, following procedure **(C)**, dry THF was added calculating a final dilution of 3.1 mL/mmole of alkenyl halide. Afterwards, 1.2 equivalents of TMEDA, 1 equivalent of alkenyl halide and 1.2 equivalents of organozinc halide, were added to the solution, that was then warmed up to reflux until completion. Reaction was monitored by GC. At last, 10 mL of saturated solution of NH_4Cl were added, to quench the reaction. The aqueous phase was extracted 3 times with diethyl ether, the collected organic fractions were dried over sodium sulphate, filtered and concentrated at reduced pressure. The crude was purified by flash chromatography.

*4.5.1 Methyl (Z)-dodec-7-enoate (**9**):*

Following general procedure (D), to a flask containing 1 mol% of $PdCl_2(PPh_3)_2$ (10.5 mg), (or the supported version of the catalyst, as reported in Results and Discussion), (Z)-1-bromohex-1-ene (**5**) (1.5 mmol, 200 µL), 1mL of THF and 246 µL of TMEDA (1.65 mmol) were added. The synthesized organozinc intermediate, 5-methoxycarbonyl-1-pentylzinc bromide (**7**), was added as a THF solution (4.1 mL of a 0.40M solution, 1.65 mmol) previously titrated following general procedure (C). The reaction was warmed up at reflux for 3 hours and quenched as reported in general procedure (D). The product was isolated by flash chromatography on silica gel (Hex/Et_2O, 95:5), as a colorless oil with the yields reported in Table 4. Spectroscopic data in agreement with literature.[44] ^{1}H NMR (600 MHz, CDCl3) δ 5,33 (dt, *J* = 10 Hz, 2H), 3.66 (s, 3H), 2.30 (t, *J* = 7 Hz, 2H), 1.65-1.61

(m, 4H), 1.36-1.30 (bs, 8H), 0.89 (t, J = 7 Hz, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 174.3, 130.2, 129.5, 51.5, 34.1, 31.9, 29.4, 28.8, 27.0, 26.9, 24.9, 22.4, 14.0; IR (neat): 3003, 2930, 2852, 1740, 1460, 1432, 1376, 1359, 1315, 1258, 1200, 1172, 1116, 1077, 1013, 912, 733 cm^{-1}; MS, m/z (%): 212(7), 180(30), 138(45), 123(24), 110(24), 96(57), 74(82), 55(100), 41(55)

*4.5.2. Methyl (Z)-hexadec-7-enoate (**10**):*

Following general procedure (D), to a flask containing 0.05 mol% of $PdCl_2(PPh_3)_2$ (1.6 mg), (or the supported version of the catalyst, as reported in Results and Discussion), (Z)-1-bromodec-1-ene (**6**) (4.5 mmol, 902 µL), 4.3 mL of THF and 738 µL of TMEDA (5 mmol) were added. The synthesized organozinc intermediate, 5-methoxycarbonyl-1-pentylzinc bromide (**7**), was added as a THF solution (9.6 mL of a 0.40M solution, 5.41 mmol) previously titrated following general procedure (C). The reaction was warmed up at reflux for 3 hours and quenched as reported in general procedure (D). The product was isolated by flash chromatography on silica gel (Hex/Et_2O, 95:5), as a colorless oil with the yields reported in Table 5. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5,33 (dt, J = 10,8 Hz, 2H), 3,66 (s, 3H), 2,30 (t, J = 7,6 Hz, 2H), 2,00-1,95 (m, 4H), 1,63-1,58 (m, 2H), 1,39-1,20 (m, 16H), 0,87 (t, J = 7,0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 130.3, 129.5, 51.6, 34.1, 32.0, 29.8, 29.6, 29.5, 29.4, 28.9, 27.3, 27.1, 25.0, 22.8, 14.3 IR (neat): 2924, 2852, 1743, 1460, 1435, 1362, 1317, 1253, 1200, 1172, 1119, 1079, 1010, 727 cm^{-1}; MS, m/z (%): 268(8), 236(41), 194(33), 110(35), 96(68), 74(85), 69(64), 55(100), 41(69). Anal. Elem., Calc. per C17H32O2: C 76,06%; H 12,02%. Found C 76,11%; H 12,06%

*4.5.3. Methyl (Z)-hexadec-11-enoate (**11**):*

Following general procedure (D), to a flask containing 0.05 mol% of $PdCl_2(PPh_3)_2$ (1.6 mg), (or the supported version of the catalyst, as reported in Results and Discussion), (Z)-1-bromohex-1-ene (**5**) (4.5 mmol, 602 µL), 4.4 mL of THF and 738 µL of TMEDA (5 mmol) were added. The synthesized organozinc intermediate, (10-methoxy-10-oxodecyl) zinc(II) bromide (**8**), was added as a THF solution (9.5 mL of a 0.57M solution, 5.41 mmol) previously titrated following general procedure (C). The reaction was warmed up at reflux for 3 hours and quenched as reported in general procedure (D). The product was isolated by flash chromatography on silica gel (Hex/Et_2O, 95:5), as a colorless oil with the yields reported in Table 5. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5,34 (t, J = 5,5 Hz, 2H), 3,66 (s, 3H), 2,29 (t, J = 7,4 Hz, 2H), 2,05-1,95 (m, 4H), 1,65-1,57 (m, 2H), 1,36-1,21 (m, 16H), 0,94-0,84 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 130.0, 51.6, 34.2, 32.1, 29.9, 29.6, 29.4, 29.3, 27.3, 27.0, 25.0, 22.5, 14.1. IR (neat): 2924, 2852, 1743, 1460, 1435, 1376, 1359, 1256, 1197, 1169, 1116, 1099, 1018, 915, 876, 803, 730 cm^{-1}; MS, m/z (%): 268(9), 235(40), 194(28), 152(24), 96(45), 74(55), 69(59), 55(100), 41(49). Anal. Elem., Calc. per C17H32O2: C 76,06%; H 12,02%. Found C 76,10%; H 12,07%

*4.7. General procedure **(F)** for female olive fruit fly breeding and preparation of extraction samples*

Insects used in this study were obtained from pupae collected in a Tuscan olive-mill during October 2016. The flies were reared following the method described by Carpita et al. **(13)**.

*4.8. General procedure **(G)** for identification and quantification of the sex-specific components in female olive fruit fly rectal glands*

The solutions extracted from virgin female rectal glands, at 1, 4, 9, 13, 17, and 21 days after the fly emergence (December 2015) were analysed by GC and GC/EI-MS. For each age, 3 samples (each one from 10 virgin females) were analysed in triplicates. Both GC and GC/EI-MS were performed with splitless injection technique, injecting 3 µl of hexane solution of the samples, using helium as carrier (1mL/min). The programmed temperature for GC and GC/EI-MS was set as following: 1 min. at 40°C, temp. ramp at 10°C/min until 250°C, 5 min. at 250°C, temp. ramp at 20 °C until 280°C, 30 min. at 280°C. For the comparison of retention times a slower program was used: 1 min. at 40°C, temp. ramp at 10°C/min until 200°C, temp. ramp at 6°C until 280°C, 30 min. at 280°C. Compounds were identified correlating mass spectra and retention times, over two different columns, with commercially available standards or with synthesized molecules. The quantification of the identified compounds was done through GC, using absolute calibration curves obtained from pure standard injections at five different concentrations (3 times), in range between 0.5 and 17.3 mg/L.

Acknowledgements

This research was partially funded by Ministry of Education, University and Research, Italy (PRIN 2007). G.A. is grateful to GABA Therwil GmbH - A Colgate-Palmolive Company for instruments donation.

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