

2种不同成熟度杨桃对橘小实蝇的产卵影响 及挥发物成分比较分析

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摘要:【目的】探讨2种不同成熟度杨桃(黄熟杨桃和绿熟杨桃)对橘小实蝇雌虫产卵量的影响并分析2者挥发物成分的差异。【方法】采用产卵杯法比较分析黄熟杨桃和绿熟杨桃对橘小实蝇雌虫产卵量的差异, 同时采用固相微萃取技术(solid phase microextraction, SPME)和气相色谱-质谱联用技术(GC-MS)对2者的挥发性化学成分进行种类和含量的比较分析。【结果】黄熟杨桃对橘小实蝇雌虫的产卵量均显著高于绿熟杨桃。2种不同成熟度杨桃的挥发性物质在种类和含量上存在明显差异, 黄熟杨桃中共检测出30种气味挥发性物质, 而绿熟杨桃中共检测出24种气味物质, 且有多种化合物在黄熟杨桃中存在而在绿熟杨桃中未检测出。除此之外, 黄熟杨桃中的一些物质, 如己酸甲酯、乙酸-反-2-己烯酯、庚酸甲酯、罗勒烯异构体混合物、辛酸甲酯、β-紫罗酮和4,6(Z),8(Z)-大柱三烯这7种物质的含量均明显高于绿熟杨桃。【结论】己酸甲酯、乙酸-反-2-己烯酯、庚酸甲酯、罗勒烯异构体混合物、辛酸甲酯、4,6(Z),8(Z)-大柱三烯、β-紫罗酮这7种物质可能是黄熟杨桃引诱橘小实蝇产卵的活性物质。

关键词: 橘小实蝇; 固相微萃取; 气质联用; 产卵杯

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Effect of different maturities of starfruit on the ovipositing of *Bactrocera dorsalis* and comparative analysis of volatile components

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Abstract:【Objective】*Bactrocera dorsalis* (Hendel) (Tephritidae: Diptera) is a major pest of fruit crops. It is highly polyphagous and causes economic losses to a wide range of fruit crops, consisting of more than 250 species of fruit and vegetables. The insect has a strong reproduction capability, with each female able to produce 400–1 800 eggs. Female *B. dorsalis* lay their eggs in mature fruits, while they are still on the tree, which causes the fruit to rot and fall in advance. Thus, oviposition is the main cause of serious harm to fruits and vegetables by the female *B. dorsalis*. Therefore, oviposition from the female *B. dorsalis* will be the key entry point to control any potential harm from *B. dorsalis*. At present, the control method to use against *B. dorsalis* is primarily induced and chemical control. However, *B. dorsalis* have developed a strong resistance to a variety of chemical pesticides. Therefore, the control effect of chemical pesticides is greatly reduced. Pesticides can seriously affect environmental safety. But the effective attractant, methyl eugenol, is primarily used to lure and control the male *B. dorsalis*. It has been reported that methyl eugenol is not only a safety hazard to the ecological balance, but also may induce liver cancer in mammals. Therefore, the search for a new attractant in the host fruit will be an effective way to control *B. dorsalis*.

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The attractive effects of various host fruit volatiles on the female flies have been reported. Starfruit is one of the important hosts of *B. dorsalis*. There previously has been no report on the research of the oviposition lure of starfruit to female *B. dorsalis*. In this paper, the effect of two different maturities of starfruit (yellow ripe starfruit and green ripe starfruit) on the oviposition of female *B. dorsalis* and the differences of their volatile components of yellow and green ripe starfruit were compared and analyzed using a solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS). It was also speculated that the yellow ripe starfruit contains the volatile substances that induce the oviposition activity of the female *B. dorsalis*. The paper will provide a scientific basis for the exploration of the oviposition attractant to the female *B. dorsalis*. **【Methods】**The difference in the amount of eggs laid by female *B. dorsalis* between yellow and green ripe starfruit were analyzed by using a spawning cup. The spawning cup was made of disposable plastic and used to fill holes with insect needles. Two spawning cups were placed in the diagonals of the feeding cage and in the bottom of each spawning cup was placed an equal amount of yellow and green ripe startfruit as a treatment and control, respectively. In the experiment, 1, 5, 10, 20 and 40 g yellow and green ripe startfruits in each spawning cup were tested as different treatments. Each treatment was repeated 3 times, and after 24 h, the spawning cup was removed to check the amount of eggs laid by the female *B. dorsalis*. The yellow and green ripe starfruit were sealed in 250 mL sample bottles for the gas volatilization process. After 15 min, the volatiles from the yellow and green ripe starfruit were collected by using the solid phase microextraction (SPME) sampling technique. The comparative analysis of the volatile components from yellow and green ripe starfruit was identified by applying the gas chromatographic-mass spectrometric (GC-MS) method. All test data were statistically analyzed and processed using Excel, and SPSS 22 software to determine the significance of the test data. **【Results】**The amounts of eggs on the yellow ripeness starfruit were significantly higher than those on the green ripe starfruit in every treatment (1, 5, 10, 20 and 40 g). It was determined that the yellow ripe starfruit had significant oviposition attractant activity for the female *B. dorsalis*. The amount of eggs was increased with the increase of the weight of the yellow ripe starfruit. When yellow ripe starfruit was 1, 5, 10, 20 and 40 g, the oviposition capacity was (5.7±0.7), (8.0±0.6), (24.0±1.2), (45.0±1.7) and (48.0±1.2) grain per cup, respectively. The results showed that the chemical composition and content of the volatile from yellow and green ripe starfruit were different. 30 volatile compounds were detected in yellow ripe starfruit, while only 24 volatile compounds were detected in green ripe starfruit. The volatile chemical components of the yellow and green ripe starfruit were primarily esters and caryophyll derivatives. Esters were primarily methyl hexanate, 2-Hexen-1-ol, acetate and methyl heptanate. The content of 2-Hexen-1-ol, acetate was the largest, accounting for 68%-84% of the total ester. The derivatives of the ring caramenes were primarily of megastigma-4, 6(Z), 8(Z)-triene, which could be a characteristic compound of starfruit. A variety of compounds in yellow ripe starfruit were not detected in green ripe starfruit. In addition, the contents of these 7 substances (methyl hexanoate; 2-Hexen-1-ol, acetate; beta-cis-ocimene; methyl octanoate; beta-Ionone; methyl heptanoate; megastigma-4, 6(Z), 8(Z)-triene) in yellow ripe starfruit were significantly higher than those in green ripe starfruit. **【Conclusion】** Yellow ripe starfruit can induce the female *B. dorsalis* to lay eggs. The results showed that the yellow ripe starfruit had some specific chemical substances that induced the female *B. dorsalis* to oviposition. The contents of methyl hexanoate; 2-Hexen-1-ol, acetate; beta-cis-ocimene; methyl octanoate; methyl heptanoate; beta-Ionone and megastigma-4, 6(Z), 8(Z)-triene in yellow ripe starfruit were significantly higher than those in green ripe starfruit. So, it can be speculated that these compounds possibly were attractive components that entice female *B. dorsalis* to oviposition.

Key words: *Bactrocera dorsalis*; SPME; GC-MS; Spawning cup

橘小实蝇(*Bactrocera dorsalis*)属双翅目实蝇科实蝇属,是一种危害40多个科250多个种果蔬的毁灭性害虫。该虫繁殖能力极强,每头雌虫可产400~1 800粒卵,田间危害率严重时为80%~90%^[1-2]。橘小实蝇危害方式主要是以雌虫产卵于果皮下,幼虫孵化后直接取食果肉,从而导致果实烂果、落果,可见产卵是橘小实蝇造成果蔬严重危害的主要根源。因此,控制其产卵将是控制橘小实蝇危害的有效措施^[3]。

目前橘小实蝇的防治方法主要有诱杀和化学防治。但橘小实蝇已对多种化学农药产生严重抗药性,因此不仅化学防治效果降低,而且由于农药抗性而被迫增加药剂剂量和防治次数,严重影响果树的绿色生产和环境安全^[4]。诱杀主要使用类性引诱剂甲基丁香酚、水解蛋白和植物提取物等,但高效诱杀剂甲基丁香酚主要用于引诱橘小实蝇雄虫。已有报道发现,甲基丁香酚不仅对生态平衡存在安全隐患,还有可能诱导哺乳动物产生肝癌肿瘤^[5]。因此,从寄主水果中寻找新型引诱剂将是对橘小实蝇进行绿色可持续治理的有效途径。

研究表明,多种寄主果实挥发物对实蝇雌虫具有引诱作用^[6-9]。杨桃是橘小实蝇的重要寄主之一,目前有关杨桃挥发物对橘小实蝇产卵引诱等方面的研究内容尚未见报道。笔者测定了黄熟和绿熟2种不同成熟度杨桃对橘小实蝇雌虫产卵的影响,并利用固相微萃取-气相色谱-质谱联用技术(SPME-GC-MS)对这2种不同成熟度杨桃的挥发物进行了比较分析,以初步推测黄熟杨桃中对橘小实蝇雌虫产卵具有引诱活性的挥发性物质,为探索开发新型橘小实蝇雌虫产卵引诱剂提供科学依据。

1 材料和方法

1.1 供试材料

供试虫源:橘小实蝇(*B. dorsalis*)幼虫采用人工饲料进行室内饲养。饲养条件为:温度(27±1)℃,相对湿度50%~70%,光周期L(光照):D(黑暗)=14 h:10 h。选用饥饿处理的产卵期雌虫作为供试虫源。

试验用杨桃(品种为‘台湾红肉杨桃’)购自市场,待洗净后用于测试。根据杨桃成熟度分为黄熟杨桃和绿熟杨桃2组。

1.2 主要仪器设备

自制产卵杯:该自制产卵杯采用一次性塑料水

杯,使用前用昆虫针在塑料杯周围等间距均匀扎满产卵孔。气相色谱-质谱联用仪:型号TRACE GC-2000 GC-MSTM,购自美国Thermo Finnigan公司。顶空微萃取仪:由SPME手动进样手柄和50/30 μm的DVB/CAR/PDMS微萃取头组成,购自Supelco公司。

1.3 方法

1.3.1 杨桃果肉挥发物对橘小实蝇产卵量的影响 首先将20对性成熟的橘小实蝇(雌:雄=1:1)接入50 cm×50 cm×40 cm的养虫笼中,人工饲料(*m* 酵母粉:*m* 蔗糖=1:1)供补充营养。在养虫笼的对角线位置分别放置2个自制产卵杯,每个产卵杯底部分别放置等量的黄熟与绿熟杨桃果肉作为处理和对照,试验共设置5个处理,每个产卵杯中分别放置1、5、10、20、40 g的黄熟和绿熟杨桃果肉作为不同处理,每个处理3次重复,24 h后取出产卵杯检查产卵量。试验在温度(27±1)℃、光周期14 h光:10 h暗、相对湿度50%~70%的室内进行。

1.3.2 杨桃挥发物的收集 选取黄熟与绿熟杨桃,洗净、晾干表面水分,在电子天平上称取约20 g,将其密封于250 mL的顶空样品瓶中进行气体挥发平衡15 min,平衡后采用50/30 μm的固相微萃取纤维DVB/CAR/PDMS进行顶空萃取30 min,萃取收集后于250 ℃解吸3 min进样。

1.3.3 杨桃挥发物的GC-MS测定 挥发物在气相色谱-质谱联用仪(Agilent公司)上进样,石英毛细管柱HP-5(30 m×0.25 mm),利用计算机标准质谱图库(NIST)并辅助以人工识别确定化合物,以质谱离子峰面积百分数表示各成分的相对含量。升温程序:柱起始温度45 ℃,保持2 min,以5 ℃·min⁻¹升至180 ℃,保持2 min,然后以10 ℃·min⁻¹升至220 ℃,保持5 min;进样口温度220 ℃。载气为He,流速1 mL·min⁻¹;质谱分析条件:电子轰击(EI)离子源;电子能量70 eV;电子倍增器电压350 V;质量范围扫描35~335 amu。

1.4 数据分析与处理

所有试验数据均采用Excel进行统计与处理,采用SPSS 22软件进行差异显著性分析。

2 结果与分析

2.1 杨桃对橘小实蝇产卵量的影响

在自制产卵杯中分别放置黄熟和绿熟2种不同成熟度的杨桃果肉,测试其对橘小实蝇产卵量的影

响。由表1可见,黄熟杨桃上橘小实蝇雌虫的产卵量均显著高于对照(绿熟杨桃),表明黄熟杨桃对橘小实蝇具有显著的产卵引诱活性,且橘小实蝇的产卵量随着黄熟杨桃果肉质量增加呈增加趋势,当黄熟杨桃为1 g时,雌虫产卵量最少,为(5.7±0.7)粒/杯;而当黄熟杨桃为20 g时,产卵量最多,为(48.0±1.2)粒/杯(表1)。

2.2 杨桃挥发物的GC-MS指纹图谱分析

应用GC-MS对黄熟与绿熟杨桃的挥发物进行检测,图1和图2分别为黄熟和绿熟杨桃挥发物的总离子流程图(TIC)。TIC图显示分离效果好,背景干扰小。对总离子流图中的各色谱峰经质谱扫描后得

表1 杨桃对橘小实蝇产卵量的影响

Table 1 Influence of starfruit on oviposition of *B. dorsalis*

杨桃鲜质量 Fresh weight of starfruit/g	产卵量/(粒/杯) Oviposition/(Grain per cup)	
	黄熟杨桃 Yellow ripeness starfruit	绿熟杨桃 Green ripeness starfruit
1	5.7±0.7 a	3.3±0.3 *
5	8.0±0.6 a	5.0±0.6 *
10	24.0±1.2 b	7.7±0.3 *
15	45.0±1.7 c	7.0±0.6 *
20	48.0±1.2 c	6.7±0.3 *

注: *表示黄熟与绿熟杨桃上的产卵量差异显著($P < 0.05$)。同列数据中不同小写字母表示邓肯氏多重比较差异显著($P < 0.05$)。

Note: * indicates significant difference between yellow ripe starfruit and green ripe starfruit ($P < 0.05$). Different small letters in same row indicate significant difference by Duncan's test at 0.05 level.

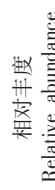


图1 黄熟杨桃挥发物的总离子流

Fig. 1 Total ion current of volatile from yellow ripeness starfruit

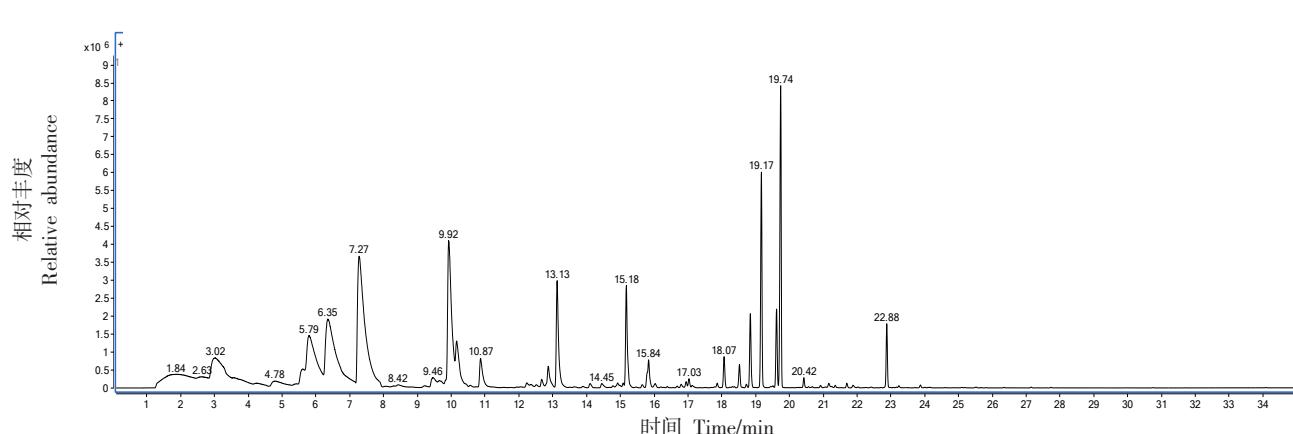


图2 绿熟杨桃挥发物的总离子流

Fig. 2 Total ion current of volatile from green ripeness starfruit

到质谱图,经质谱数据系统检索和对照碎片峰分度对各化学成分进行了定性,并利用总离子流色谱峰的峰面积对各化学成分进行了相对定量。

2.3 2种不同成熟度杨桃挥发物的比较分析

采用相应质谱图计算机检索的方法对黄熟和绿

熟杨桃挥发物进行检索,共鉴定出41种化学成分,用面积归一法计算它们的相对含量。由表2可知,黄熟杨桃和绿熟杨桃挥发物的化学成分不完全相同,并且对于同一种化合物2者在含量上也存在很大差异。黄熟杨桃中共检测出30种气味挥发性物

表 2 杨桃挥发性化学物质及其相对含量
Table 2 Volatile chemical from starfruit and their relative contents

保留时间 Retention time/min	化合物 Compounds	CAS 编号 CAS registry number	相对含量 Relative content/%	
			黄熟杨桃 Yellow ripeness starfruit	绿熟杨桃 Green ripeness starfruit
5.62	乙基苯 Ethylbenzene	100-41-4	0.41	-
5.79	间二甲苯 Benzene, 1,3-dimethyl-	108-38-3	7.97	14.28
6.35	苯乙烯 Styrene	100-42-5	12.10	29.93
7.27	己酸甲酯 Methyl hexanoate	106-70-7	15.81	-
8.42	反式-2-己烯酸甲酯 2-Hexenoic acid, methyl ester, (E)-	13894-63-8	0.18	-
9.46	正己酸乙酯 Hexanoic acid, ethyl ester	123-66-0	0.74	0.71
9.92	乙酸-反-2-己烯酯 2-Hexen-1-ol, acetate	10094-40-3	9.19	-
10.16	庚酸甲酯 Methyl heptanoate	106-73-0	2.99	-
10.56	(E)-β-罗勒烯 Trans-beta-Ocimene	3779-61-1	0.08	-
10.86	罗勒烯 Beta-Ocimene	13877-91-3	-	0.12
10.86	罗勒烯异构体混合物 Beta-cis-Ocimene	3338-55-4	1.38	-
11.94	3,4,5-三甲基甲苯 Benzene,1,2,3,5-tetramethyl-	527-53-7	0.02	-
12.23	苯甲酸甲酯 Benzoic acid, methyl ester	93-58-3	0.22	-
12.36	庚酸乙酯 Heptanoic acid, ethyl ester	106-30-9	-	0.14
12.67	丙酸-反-2-己烯酯 2-Hexen-1-ol, propanoate, (E)-	53398-80-4	0.30	0.71
13.13	辛酸甲酯 Methyl octanoate	111-11-5	3.86	0.15
13.64	2,6-二甲基-2,4,6-辛三烯 2,4,6-Octatriene,2,6-dimethyl-	673-84-7	0.05	-
14.45	反式-2-辛烯甲酯 2-Octenoic acid, methyl ester, (E)-	7367-81-9	0.19	-
14.78	萘 Naphthalene	91-20-3	0.06	0.04
14.92	丁酸-3-己烯酯(E) Butanoic acid, 3-hexenyl ester, (E)-	53398-84-8	-	0.31
15.08	丁酸己酯 Butanoic acid, hexyl ester	2639-63-6	0.11	0.22
15.17	N-丁酸-反-2-己烯酯 Butanoic acid, 2-hexenyl ester, (E)-	53398-83-7	2.92	4.01
17.03	7(E),9,13-大柱三烯 Megastigma-7(E),9,13-triene	81983-67-7	-	0.02
17.12	1,2,3,4-四氢-1,1,6-三甲基萘 Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	475-03-6	0.09	-
17.69	茴香脑 Anethole	104-46-1	-	0.03
17.86	反-2-己烯基异戊酸酯 Trans-2-Hexenyl isovalerate	68698-59-9	-	0.17
18.06	2,6,10,10-四甲基-1-氧杂螺[4.5]癸-6-烯 1-Oxaspiro[4.5]dec-6-ene,2,6,10,10-tetramethyl-	36431-72-8	0.70	-
18.72	癸酸甲酯 Decanoic acid, methyl ester	110-42-9	0.08	-
19.16	4,6(E),8(E)-大柱三烯 Megastigma-4,6(E),8(E)-triene	51468-86-1	-	0.57
19.51	1,1,5 三甲基-2-二氢萘 1,1,5-Trimethyl-1,2-dihydronaphthalene	1000357-25-8	0.04	-
19.74	4,6(Z),8(Z)-大柱三烯 Megastigma-4,6(Z),8(Z)-triene	71186-25-9	6.32	0.96
20.42	N-己酸-反-2-己烯酯 Hexanoic acid, 2-hexenyl ester, (E)-	53398-86-0	-	0.21
20.67	十四烷 Tetradecane	629-59-4	0.02	0.04
20.91	2-(甲氨基)苯甲酸甲酯 Benzoic acid, 2-(methylamino)-, methyl ester	85-91-6	0.05	-
21.07	(1S,2R,5S)-2,6,6,8-四甲基三环[5.3.1.01.5]十一碳-8-烯 1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	469-61-4	-	0.02
21.16	β-二氢紫罗兰酮 2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	17283-81-7	0.12	0.04
21.25	反式石竹烯 Caryophyllene	87-44-5	0.03	0.04
21.88	2E)-2-己烯酸 (2E)-2-己烯-1-酯 2-Hexenoic acid, 2-hexenyl ester, (E,E)-	54845-28-2	-	0.05
22.87	β-紫罗酮 Beta-Ionone	79-77-6	1.27	0.45

注：“-”表示未检测到该化合物。

Note: “-” indicates that the compound was not detected.

质,而绿熟杨桃中共检测出24种气味物质,且有多种化合物在黄熟杨桃中存在而在绿熟杨桃中未检测出。如乙基苯、己酸甲酯、反式-2-己烯酸甲酯、乙酸-反-2-己烯酯、庚酸甲酯、(E)- β -罗勒烯、罗勒烯异构体混合物、3,4,5-三甲基甲苯、苯甲酸甲酯、2,6-二甲基-2,4,6-辛三烯、反式-2-辛烯甲酯、1,2,3,4-四氢-1,1,6-三甲基萘、2,6,10,10-四甲基-1-氧杂螺[4.5]癸-6-烯、癸酸甲酯、1,1,5三甲基-2-二氢萘和2-(甲氨基)苯甲酸甲酯等17种化合物。此外,还有一些化合物在黄熟杨桃中的含量明显高于绿熟杨桃,含量高于0.8%。如己酸甲酯、乙酸-反-2-己烯酯、庚酸甲酯、罗勒烯异构体混合物、辛酸甲酯、4,6(Z),8(Z)-大柱三烯、 β -紫罗酮这7种化合物在黄熟杨桃中的含量均显著大于绿熟杨桃。

3 讨 论

寄主植物是影响昆虫生长发育和繁殖的重要因素^[10]。Ishiguri等^[11]研究发现,寄主果实的营养物质对幼虫生长发育有影响。笔者比较分析了2种不同成熟度杨桃寄主果实对橘小实蝇雌虫的产卵影响,结果表明,橘小实蝇雌虫在黄熟杨桃上的产卵量也显著高于绿熟杨桃。所以,可能是由于黄熟杨桃寄主果实内的营养物质更加适合橘小实蝇幼虫生长发育,导致橘小实蝇雌虫选择在黄熟杨桃上产卵。同时也说明黄熟杨桃中具有引诱橘小实蝇雌虫产卵的特异性化学物质。笔者利用气相色谱和质谱联用对杨桃挥发性物质检测可知,杨桃果实的挥发性化学成分主要是酯类及环香叶烯衍生物,其中酯类物质的种类和含量最多,酯类物质以己酸甲酯、乙酸-反-2-己烯酯、庚酸甲酯为主,其中乙酸-反-2-己烯酯含量最多,占全部酯类的68%~84%。环香叶烯衍生物中以4,6,8-大柱三烯为主,对于大柱三烯,目前在其他种类的水果香气研究中暂未见有报道,它可能是杨桃的特征性化合物,这与国外的报道相同^[12~13]。笔者通过对黄熟和绿熟杨桃挥发物化学成分的比较分析可知,黄熟杨桃中的己酸甲酯、乙酸-反-2-己烯酯、庚酸甲酯、罗勒烯异构体混合物、辛酸甲酯、 β -紫罗酮和4,6(Z),8(Z)-大柱三烯7种物质的含量均较绿熟杨桃有明显增加,其中部分物质或其类似物质已报道对实蝇具有引诱作用。据报道,己酸甲酯对橘小实蝇具有引诱作用^[14]。陈玲等^[15]研究表明, β -紫罗酮可吸引橘小实蝇。汪佳晴

等^[16]研究表明, β -罗勒烯和顺-3-己烯乙酸酯单体对柳黑毛蚜(*Chaitophorus saliniger* Shinji)均具有较强的引诱效果。范锦胜等^[17]发现,已交配草地螟雌虫对顺-3-己烯乙酸酯表现出较强的趋性。唐睿等^[18]报道了反-2-己烯乙酸酯引起美国白蛾(*Hyphantria cunea*)雄虫的触角电位反应最强。这些被证实对橘小实蝇有引诱活性的物质与上述在黄熟杨桃挥发物中检测出的7种含量较高的化合物均一致或结构相似。而其他含量较高化合物对橘小实蝇的引诱活性笔者将后续研究。这些活性化合物的确定对于开发新型橘小实蝇引诱剂将具有重要的理论和实践意义。

4 结 论

橘小实蝇雌虫选择在黄熟杨桃上产卵,表明黄熟杨桃中具有引诱橘小实蝇雌虫的特异性化学物质。己酸甲酯、乙酸-反-2-己烯酯、庚酸甲酯、罗勒烯异构体混合物、辛酸甲酯、4,6(Z),8(Z)-大柱三烯、 β -紫罗酮7种化合物在黄熟杨桃中的含量均显著大于绿熟杨桃。推测这7种物质可能是黄熟杨桃引诱橘小实蝇雌虫产卵的主要活性物质。

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