

7 种挥发性化合物对柑橘木虱引诱效果的评价

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摘要 柑橘黄龙病对全球的柑橘产业造成了严重的经济损失,其传播媒介主要为柑橘木虱。在前期研究中利用柑橘木虱对 4 种芸香科植物的取食偏好结合代谢组学分析筛选出一批可能对柑橘木虱具有引诱效果的挥发性化合物,本研究鉴定了邻氨基苯甲酸甲酯、癸酸、正辛醇、左旋香芹酮、2-环己烯酮、反式-1,2-环己二醇、吡嗪等 7 种挥发性化合物在 0、0.1%、1%、10% 4 个浓度和 10、20、30、40、50、60 min 6 个持续时间下对柑橘木虱的引诱效果,并比较了其中 5 种脂溶性挥发物在各自最优浓度下的引诱效果。试验结果表明,7 种挥发物对柑橘木虱都有引诱效果,但是引诱效果与挥发物浓度及持续时间有关。吡嗪、左旋香芹酮、正辛醇、2-环己烯酮、癸酸、邻氨基苯甲酸甲酯和反式-1,2-环己二醇的最佳引诱浓度分别为 0.1%、1%、1%、1%、10%、10%、10%。六臂嗅觉仪试验结果显示 5 种脂溶性挥发物中 1% 左旋香芹酮的引诱效果高于 10% 癸酸、10% 邻氨基苯甲酸甲酯、1% 2-环己烯酮、1% 正辛醇及对照(矿物油),但与其他 4 种挥发物没有显著差异。研究结果可为柑橘木虱引诱剂的开发提供参考,促进柑橘黄龙病的绿色防控。

关键词 黄龙病; 亚洲柑橘木虱; 植物次生代谢物; 引诱剂

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Evaluation of attraction effect of seven volatile compounds on *Diaphorina citri* Kuwayama

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Abstract Huanglongbing has caused great economic loss to the citrus industry globally, which is mainly spread by *Diaphorina citri* Kuwayama. Based on host plant preference and metabolic profiling, a number of volatile organic compounds (VOCs) that may attract *D. citri* have been identified from four Rutaceae host plants in our previous study. Here seven VOCs were selected and their attractive effect on *D. citri* was analyzed at four concentrations (0, 0.1%, 1%, 10%) and six duration (10, 20, 30, 40, 50, 60 min), namely methyl anthranilate, decanoic acid, *n*-octanol, *trans*-1,2-cyclohexanediol, pyrazine, *l*-carvone and 2-cyclohexen-1-one. Meanwhile, the responses of *D. citri* to five fat-soluble VOCs at their respective best attracting concentration were compared. The results revealed that these seven VOCs were all attractive to *D. citri* and the attracting effects varied depending on concentration and experiment duration. The best attracting concentrations of pyrazine, *l*-carvone, *n*-octanol, 2-cyclohexen-1-one, decanoic acid, methyl anthranilate and *trans*-1,2-cyclohexanediol were 0.1%, 1%, 1%, 1%, 10%, 10% and 10%, respectively. Six-armed olfactometer detection suggested that 1% *l*-carvone was more attractive to *D. citri* than 10% decanoic acid, 10% methyl anthranilate, 1% 2-cyclohexen-1-one, 1% *n*-octanol and the control (mineral oil), but there was no significant difference between *l*-carvone and the other 4 volatiles. Our result may provide alternatives for the development of environment-friendly *D. citri* attractant and promote the green prevention and control of citrus Huanglongbing.

Key words Huanglongbing; Asian citrus psyllid; plant secondary metabolite; attractant

柑橘黄龙病(Huanglongbing, HLB)严重威胁着全球柑橘产业的健康发展,柑橘木虱 *Diaphorina citri* Kuwayama 是黄龙病致病菌的主要传播媒介^[1]。积极施用杀虫剂可以控制柑橘木虱,但也可能造成耐药性、环境污染、农药残留、危害天敌等诸多问题^[2]。因此,许多环境友好的柑橘木虱防控方法被开发,如精油、寄生蜂和昆虫病原真菌,以及黄色粘虫板^[3-6]。

以挥发性化合物为模板开发引诱剂或驱避剂被认为是保护柑橘类植物免受 HLB 侵害的潜在途径,被称为柑橘木虱防治的“push-pull”策略,是柑橘木虱防治的国际趋势^[7]。现已报道了大量对柑橘木虱有驱避作用的化学物质。例如,二甲基三硫化物和二甲基二硫化物以 1:1 比例混合会抑制柑橘木虱对宿主的选择^[8]。(E)- β -石竹烯,一种从番石榴中鉴定出的挥发性化合物,对柑橘木虱也具有排斥作用^[9]。来自非寄主腰果的萜类也降低了寄主植物挥发物对柑橘木虱的吸引^[10]。

植物被害虫取食后可导致挥发性化合物的总排放量增加,特别是刺激单萜和倍半萜的异戊二烯/萜烯途径,从而释放大量的诱导性化合物^[11-12],可能是感病植株较健康植株更吸引柑橘木虱的原因之一。同时,宿主植物产生的挥发性化合物是柑橘木虱取食和产卵选择的关键因素之一^[2, 13]。因此,可以利用对柑橘木虱有引诱效果的宿主植物中的挥发性化合物开发引诱剂。

前期研究我们利用顶空气相色谱法分析了崇义野橘、九里香 *Murraya exotica* L.、甜橙‘赣南早’ *Citrus sinensis* Osbeck ‘Gannanzao’和野金柑 *Fortune hindisii* Swingle 嫩芽中代谢产物的差异,因为柑橘木虱对崇义野橘具有明显的取食偏好性,所以崇义野橘中含量显著高于其他 3 种芸香科植物的次生代谢物可能具有引诱柑橘木虱的效果^[14]。本文旨在验证从中筛选的 7 种挥发性化合物对柑橘木虱的引诱效果,从而为其生物防治提供理论依据。

1 材料与方法

1.1 材料

供试虫源:柑橘木虱为赣南师范大学国家脐橙

工程技术研究中心负压实验室提供,饲养条件为:温度 $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$;光周期 L//D=14 h//10 h;相对湿度: $(60 \pm 5)\%$;寄主:九里香。由于本试验研究的是柑橘木虱整个群体的气味偏好,故没有区分试虫的年龄及性别。试虫在试验前都进行 1 h 饥饿处理。

试剂与主要仪器:98% 邻氨基苯甲酸甲酯(methyl anthranilate;CAS 号:134-20-3)、99% 癸酸(decanoic acid;CAS 号:334-48-5)、99% 正辛醇(*n*-octanol;CAS 号:111-87-5)、98% 反式-1,2-环己二醇(*trans*-1, 2-cyclohexanediol;CAS 号:1460-57-7)、99% 吡嗪(pyrazine;CAS 号:290-37-9)、99% 左旋香芹酮(*l*-carvone;CAS 号:6485-40-1)、98% 2-环己烯酮(2-cyclohexen-1-one;CAS 号:930-68-7),上海麦克林生化科技有限公司;99% 矿物油,Amresco J217;250 mL(24 * 4)四口烧瓶,建湖县军明玻璃仪器厂;BL6-300M 型六臂嗅觉仪,上海比朗仪器有限公司。

1.2 方法

1.2.1 柑橘木虱对同种挥发性化合物不同浓度梯度的选择试验

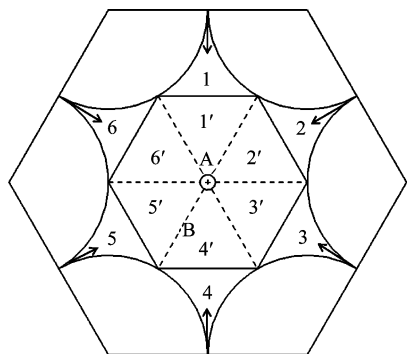
每种挥发性化合物均设置 4 个浓度梯度,分别为 0、0.1%、1%、10%,其中 0 为空白对照(即为矿物油或者超纯水)。不同浓度的邻氨基苯甲酸甲酯(V/V)、癸酸(*m*/V)、正辛醇(V/V)、左旋香芹酮(V/V)、2-环己烯酮(V/V)用矿物油配制,反式-1,2-环己二醇(*m*/V)、吡嗪(*m*/V)用超纯水配制。在四口烧瓶不同瓶口处用长度一致的橡皮管分别连接装有不同浓度挥发性化合物的味源瓶,一次放入约 40 头成虫于烧瓶底部,以过四口烧瓶的单口瓶颈为分界处,过界即视为被引诱,每隔 10 min 记录 1 次每个瓶颈柑橘木虱的数量及 4 个瓶颈柑橘木虱的总量,计算引诱率。每次试验更换未处理过的柑橘木虱,重复 3 次。因为柑橘木虱有趋光性且晚上较为活跃^[15],测试均在 19:00 后进行。

引诱率=每个瓶颈诱集木虱数量/4 个瓶颈诱集木虱总数 $\times 100\%$ 。

1.2.2 不同挥发物最优引诱浓度对柑橘木虱的引诱性比较

通过 1.2.1 确定的各挥发物的最优引诱浓度,

用六臂嗅觉仪比较 5 种脂溶性挥发物最优引诱浓度下对柑橘木虱的引诱效果。试验前将六臂嗅觉仪各臂气流流速设为 80 mL/min, 然后在各臂所对应味源瓶中放入最优引诱浓度的不同挥发物。每次试验测试 10 头成虫, 测试时逐头进行。将试虫放入嗅觉仪(图 1)后观察记录其爬行轨迹, 木虱进入初试选择区的次数记为该区对应挥发物的进入次数, 木虱在最终选择区停留 3 min 不动时记为该区对应挥发物的最终选择次数。



A: 试虫入口、气流出口; B: 气味分界线; 1~6: 最终选择区; 1'~6': 初始选择区; 箭头: 气流方向

A: Entry of *Diaphorina citri* and exit of the air; B: Bordline of the air sources; 1-6: Final choice area; 1'-6': Initial choice area; Arrow: Air current direction

图 1 六臂嗅觉仪活动室

Fig. 1 Activity room of six-armed olfactometer

1.3 数据分析

试验数据利用 SPSS 22 软件进行统计分析, 不同浓度挥发物对柑橘木虱影响和 5 种挥发物最优引诱浓度对柑橘木虱引诱效果比较的试验结果均采用单因素方差分析, 并用最小显著差数法(LSD)进行显著性差异分析。

2 结果与分析

2.1 不同浓度挥发物对柑橘木虱的影响

如表 1 所示, 在 1 h 内, 7 种挥发物在 0.1%、1%、10% 3 种浓度下的引诱率大部分都高于对照, 并存在显著性差异($P < 0.05$), 而且同一挥发物在同一浓度不同时间点的引诱率大多具有显著性差异($P < 0.05$), 说明这 7 种挥发物对柑橘木虱都有引诱效果, 但是效果与浓度及引诱持续时间有关。0.1% 左旋香芹酮和 10% 吡嗪虽然在试验 10 min 时引诱效果显著, 但是随着处理时间的延长, 引诱虫口数大幅下降, 而 1% 左旋香芹酮和 0.1% 吡嗪随着处理时间的延长引诱效果持续增加, 故将左旋

香芹酮和吡嗪的最优引诱浓度分别定为 1% 和 0.1%。不同时间点下 1% 正辛醇、10% 癸酸、10% 邻氨基苯甲酸甲酯、10% 反式-1, 2-环己二醇、1% 2-环己烯酮的引诱效果都显著高于其他浓度($P < 0.05$), 因此将对应的浓度定为该挥发物的最优引诱浓度。

2.2 5 种挥发物最优引诱浓度对柑橘木虱引诱效果

试验结果表明(表 2), 柑橘木虱进入用矿物油配制的 5 种挥发物次数与最终停留次数均高于对照矿物油, 其中柑橘木虱进入 10% 癸酸、1% 2-环己烯酮、1% 左旋香芹酮、1% 正辛醇的次数与对照存在显著性差异($P < 0.05$), 而以 1% 左旋香芹酮引诱时, 木虱最终停留区次数显著高于空白对照($P < 0.05$), 大于其他挥发物, 但没有显著性差异, 说明其可能对柑橘木虱具有最佳引诱效果。

3 讨论

植食性昆虫通过辨别寄主植物中所含次生代谢物的特殊气味进行取食, 这些特殊气味是昆虫寻找寄主植物的信号分子^[16]。我们在前期工作中从 4 个芸香科植物中筛选到 7 种挥发性化合物, 本研究测定了这 7 种挥发性化合物对柑橘木虱的引诱效果, 结果表明它们都可以引起柑橘木虱的趋性反应。这几种挥发物也可引起其他昆虫类似的趋性反应, 如正辛醇对桑天牛 *Apriona germari*、茶二叉蚜 *Toxoptem aurantii* 和巴氏新小绥螨 *Neoseiulus barkeri* 都有引诱效果^[17-19]。癸酸对亚洲玉米螟 *Ostrinia furnacalis* 初孵幼虫的取食和雄性茧蜂 *Psytalia concolor* 的寻偶都表现出引诱活性^[20-21]。邻氨基苯甲酸甲酯对近鬃秆蝇 *Thaumatomyia glabra*、墨西哥果蝇 *Anastrepha ludens*、黄胸蓟马 *Thrips hawaiiensis*、色蓟马 *T. coloratus*、葱蓟马姬小蜂 *Ceraninus menes* 都有明显的引诱效果^[22-24]。吡嗪可以影响雄性胡蜂的习性, 其衍生物可以作为胡蜂的性引诱剂^[25-27]。同时, 吡嗪是国际上普遍认可的实蝇诱引剂之一^[28]。

化合物对昆虫习性的影响因虫种及虫龄而异^[29]。本研究发现 1% *l*-香芹酮对柑橘木虱具有最佳引诱效果, 其对斑衣蜡蝉 *Lycorma delicatula* 的成虫和若虫(除 1 龄外)都具有明显的引诱效果^[30]。但是 *l*-香芹酮可能对黄蜂 *Vespula pensylvanica*、茶

表 1 7 种挥发物不同浓度及不同时间对柑橘木虱的引诱效果比较¹⁾

Table 1 Comparison of attraction effect of seven volatiles at different concentrations and duration to *Diaphorina citri*

挥发物 Volatile	浓度/% Concentration	引诱率/% Attraction rate					
		10 min	20 min	30 min	40 min	50 min	60 min
左旋香芹酮 <i>l</i> -carvone	对照 Control	(5.12±2.63)cA	(11.86±2.24)bA	(8.08±0.56)bA	(7.65±1.37)bA	(6.77±3.94)bA	(5.01±1.22)bA
	0.1	(51.62±5.83)aA	(33.01±9.16)aAB	(30.01±8.53)aAB	(30.92±9.24)aAB	(31.80±4.24)abAB	(26.80±5.37)abB
	1	(28.70±2.32)bB	(35.58±6.73)aAB	(41.58±9.44)aAB	(46.13±7.76)aAB	(42.91±6.28)aAB	(53.27±6.22)aA
正辛醇 <i>n</i> -octanol	10	(14.57±6.05)bcA	(22.33±0.75)abA	(21.67±3.99)abA	(23.00±8.20)abA	(18.52±4.61)bA	(14.92±3.93)bA
	对照 Control	(23.23±11.65)aA	(19.41±4.59)bA	(23.28±2.80)bA	(13.92±2.42)cA	(15.24±3.54)bA	(10.71±1.20)cA
	0.1	(14.31±4.81)aAB	(15.63±3.97)bAB	(11.11±5.72)bb	(27.12±4.84)bA	(20.80±2.23)bAB	(23.31±3.50)bAB
癸酸 decanoic acid	1	(31.95±4.04)aC	(38.22±2.75)aBC	(43.39±0.50)aAB	(37.90±3.17)aBC	(49.28±0.72)aA	(46.31±1.93)aaA
	10	(30.51±6.46)aA	(26.74±1.83)bAB	(22.22±5.72)bAB	(21.06±2.04)bcAB	(14.69±2.35)bb	(19.67±5.09)bcAB
	对照 Control	(15.49±11.72)bA	(10.29±4.23)bA	(12.10±3.03)cA	(8.24±1.90)dA	(7.76±1.31)cA	(8.94±0.90)cA
邻氨基苯甲酸甲酯 methyl anthranilate	0.1	(16.89±5.61)bb	(25.31±5.07)aAB	(29.15±3.20)abA	(31.57±0.46)bA	(25.76±4.61)bAB	(22.41±1.75)bAB
	1	(19.66±6.06)bA	(29.47±6.95)aaA	(21.67±1.67)bcA	(22.35±2.53)cA	(20.73±2.57)bA	(23.00±1.05)bA
	10	(47.97±6.34)aA	(34.93±5.98)aA	(37.08±2.92)aaA	(37.85±0.51)aA	(45.76±2.30)aaA	(45.65±2.35)aaA
吡嗪 pyrazine	对照 Control	(14.63±4.25)bb	(24.93±3.64)aA	(13.49±3.46)bb	(9.04±4.73)cB	(13.42±1.99)cB	(14.90±1.05)cAB
	0.1	(6.13±3.61)bc	(11.86±1.38)bbc	(13.49±3.46)bbc	(23.33±5.09)bA	(19.09±1.57)bcAB	(17.80±2.03)cAB
	1	(41.64±1.11)aaA	(26.05±6.19)abB	(23.02±6.50)bb	(21.48±0.74)bb	(23.01±3.49)bb	(25.58±2.40)bb
反式-1,2-环己二醇 <i>trans</i> -1,2-cyclohexanediol	10	(37.60±1.36)aC	(37.16±2.90)aC	(50.00±4.12)aaA	(46.15±1.93)aAB	(44.48±1.92)aABC	(41.72±1.00)abc
	对照 Control	(2.38±2.38)cB	(8.84±0.25)bAB	(17.35±1.38)bA	(16.48±5.48)bA	(11.29±2.91)cA	(8.75±2.29)cAB
	0.1	(25.87±5.87)bb	(29.29±5.62)aAB	(35.60±4.56)aAB	(39.90±5.13)aAB	(41.26±0.64)aAB	(43.19±6.81)aA
2-环己烯酮 2-cyclohexen-1-one	1	(24.13±5.87)bA	(26.52±5.30)aA	(21.58±7.26)abA	(16.22±7.52)bA	(22.75±4.52)bcA	(21.81±2.46)bcA
	10	(47.62±2.38)aaA	(35.35±1.01)aAB	(25.47±4.03)ab	(27.40±7.50)abB	(24.71±5.07)bb	(26.25±4.22)bb
	对照 Control	(14.89±2.06)bA	(11.68±2.40)bAB	(8.07±1.84)dB	(12.55±2.55)cAB	(7.41±1.15)dB	(10.25±1.56)dAB
邻氨基苯甲酸甲酯 methyl anthranilate	0.1	(11.86±1.86)bc	(25.55±1.61)aA	(23.68±0.80)cA	(13.92±1.08)cC	(19.05±1.37)cB	(16.06±1.43)cBC
	1	(33.49±1.62)aaA	(32.76±2.95)aA	(30.56±2.78)bA	(31.76±1.76)bA	(30.95±3.64)bA	(26.19±1.87)bA
	10	(39.76±2.42)aAB	(30.01±3.70)aC	(37.70±2.41)abc	(41.76±3.24)aAB	(42.59±3.56)aAB	(47.50±1.27)aA
2-环己烯酮 2-cyclohexen-1-one	对照 Control	(12.07±2.05)cA	(17.35±1.38)bA	(14.09±2.33)cA	(9.71±4.91)cA	(11.76±5.88)bA	(12.29±0.52)dA
	0.1	(28.53±4.52)bA	(24.91±1.04)abA	(30.02±0.61)bA	(32.38±3.26)bA	(27.17±1.84)abA	(26.54±1.70)bA
	1	(39.21±1.26)aAB	(35.38±5.72)ab	(39.95±1.23)aAB	(45.79±2.99)aA	(39.78±2.29)aAB	(44.97±1.90)aaA
吡嗪 pyrazine	10	(20.19±3.89)bcA	(22.35±3.52)bA	(15.93±1.72)cA	(12.12±0.83)aaA	(21.29±4.98)bA	(16.21±3.69)cA

1) 表中邻氨基苯甲酸甲酯、癸酸、正辛醇、左旋香芹酮、2-环己烯酮的对照为矿物油,反式-1,2-环己二醇、吡嗪的对照为超纯水,表中数据为平均值±标准误差;不同小写字母代表经 LSD 法检验同一时间同种挥发物不同浓度间在 $P<0.05$ 水平下差异显著,不同大写字母代表经 LSD 法检验同一浓度同一挥发物不同时间点在 $P<0.05$ 水平下差异显著。
The control of methyl anthranilate, decanoic acid, *n*-octanol, *l*-carvone and 2-cyclohexen-1-one was mineral oil. The control of *trans*-1,2-cyclohexanediol and pyrazine was ddH₂O. Data were mean±SE. Different lowercase letters indicated significant difference among different concentrations of the same volatile at the same time point by LSD test ($P<0.05$). Different uppercase letters indicated significant difference among different time points of the same volatile at the same concentration by LSD test ($P<0.05$).

表 2 柑橘木虱对 5 种挥发物的选择次数及最终停留次数比较¹⁾

Table 2 Comparison of number of choice and final choice of *Diaphorina citri* to five volatiles

挥发物 Volatile	进入次数/次 Number of choice	最终停留次数/次 Number of final choice
矿物油 Mineral oil	(0. 67±0. 33)b	(0. 67±0. 33)b
10%癸酸 10% decanoic acid	(2. 00±0. 58)a	(1. 67±0. 67)ab
1% 2-环己烯酮 1% 2-cyclohexen-1-one	(2. 33±0. 33)a	(1. 67±0. 33)ab
10%邻氨基苯甲酸甲酯 10% methyl anthranilate	(1. 67±0. 33)ab	(1. 33±0. 33)ab
1%左旋香芹酮 1% <i>l</i> -carvone	(2. 67±0. 33)a	(2. 33±0. 67)a
1%正辛醇 1% <i>n</i> -octanol	(2. 00±0. 58)a	(1. 00±0. 00)ab

1) 表中数据为平均值±标准误;不同小写字母代表经 LSD 法检验存在显著性差异($P<0.05$)。
Data were mean±SE. Different small letters indicated significant difference among different volatiles at $P<0.05$ level by LSD test.

翅蜡 *Halyomorpha halys*、淡色库蚊 *Culex pipiens* 都具有驱避效果^[30-33]。另外,正辛醇对巴氏新小绥螨的引诱作用随着浓度的增加而降低,说明化合物对昆虫习性的影响因其浓度而异^[19,28]。这与本研究发现不同浓度挥发物对柑橘木虱引诱效果存在显著差异的结果相符。

此外,这些化合物的衍生物在柑橘木虱的生物防治方面也可能具有较大的开发潜能。虽然没有查到关于反式-1,2-环己二醇直接作为昆虫引诱剂的报道,但它是蚊子产卵引诱剂的主要原料^[34]。癸酸的系列衍生物癸酸甲酯(methyl decanoate)、月桂酸甲酯(methyl dodecanoate)、肉豆蔻酸甲酯(methyl tetradecanoate)、棕榈酸甲酯(methyl hexadecanoate)、月桂酸乙酯(ethyl dodecanoate)、十四酸乙酯(ethyl tetradecanoate)对熊蜂 *Bombus diversus* 蜂王都有显著引诱效果^[35]。这些化合物的衍生物对柑橘木虱习性的影响尚不明确。

开发生物源农药如植物次生代谢物质、植物精油等天然产物对植物病虫害的生物防治具有重要的生态学意义^[15, 36]。利用植物次生代谢物质研制昆虫引诱剂符合绿色农业发展的需要,但是本研究供试挥发物在田间的引诱效果尚有待验证。

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