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柑橘木虱雌成虫对雄成虫的引诱活性及交配高峰期的研究

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摘 要：【目的】柑橘木虱（*Diaphorina citri* Kuwayama）是柑橘的主要害虫，是传播柑橘黄龙病的重要媒介，探究柑橘木虱雌雄成虫间的引诱活性及其交配高峰期，为准确提取柑橘木虱性信息素提供科学依据。**【方法】**采用Y型嗅觉仪研究柑橘木虱雌成虫对雄成虫的引诱活性。通过九里香苗饲养配对及单头的柑橘木虱，观察和探究其交配行为及规律。**【结果】**与对照相比，柑橘木虱未交配雄虫对处女雌虫的选择达到了显著水平；其他3组处理差异不显著。柑橘木虱在羽化后1~2 d无交配活动发生，羽化后3 d开始出现交尾行为，羽化后6 d交配率和重复交配率均最高，分别为24.4%和79%。除了00:30、01:00、03:00和04:30时间段，柑橘木虱均存在交配活动，但主要发生在光照期内（06:00—20:00），而且在14:30—18:30时间段内交配率较高。**【结论】**柑橘木虱交配高峰出现在羽化后第6天的成虫且在14:30—18:30，可在这一时间段对6日龄的雌虫进行柑橘木虱性信息素的提取。

关键词：柑橘木虱；引诱活性；交配规律；Y型嗅觉仪

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Study on the attractivity of female adults to the males and the peak of mating activity in *Diaphorina citri* Kuwayama

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Abstract:【Objective】*Citrus* is an important economic crop in China, and *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) is a serious pest of citrus, which is also an important medium for spreading the most devastating diseases in citrus production huanglongbing (HLB). Generally, the areas where the HLB occurs are also the distribution areas of *D. citri*. The control of *D. citri* relies largely on chemical insecticides currently, but has led to insect resistance and serious environmental problems. One of the alternatives is to use insect pheromones that have the advantages of trace, high efficiency, non-toxicity and no harm to environment and natural enemy, and play an important role in the integrated pest management. The purpose of the present work is to study the attractivity of adult *D. citri* between two sexes and the peak of mating activity of *D. citri* to provide scientific basis for accurate extraction of the sex pheromone of *D. citri*.【Methods】The Y-tube olfactometer was used to test the attractivity of adult *D. citri* between two sexes. The Y-tube olfactometer consisted of a 30-cm-long, 3-cm-diameter central tube and two 15-cm-long, 2-cm-diameter lateral arms, which were individually connected to the odor stimuli and the control through a Teflon connection. The olfactory bioassays were conducted at room temperature (27±2 ℃) from 10:00 to 20:00. Ten virgin females or males were used as lures, while the empty bottle was as the control. For each bioassay, a single insect was introduced into the central arm of Y-tube. The insect walked into one of the lateral arms when they came over 1/3 and remained for at least 60 s in that lateral arm can be recorded. If an insect did not make a choice in 5 min after being released

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into the olfactometer, it was considered as a non-responder. The mated and single older nymphal *D. citri* samples were fed on *Murraya exotica* seedlings at (27±2) ℃, 60%-70% and 14L: 10D photoperiod. *M. exotica* seedlings were pruned 8-10 cm in height 15 days before the beginning of the experiment to allow emission of flushes. Seedlings containing flushes of 1 cm in length were allocated to cover with the bigger plastic cup, which was 5.5-cm-diameter bottom, 8.5-cm-diameter top opening, and 13.5-cm-height with air hole. The bigger plastic cup (top one) and the small plastic cup (bottom one) that was 4.5-cm-diameter bottom, 6.5-cm-diameter top opening, and 8.5-cm-height with drain water hole in the bottom for planting the seedlings formed an experimental unit (cage). After that, one virgin male and one virgin female (couple) with 1, 2, 3, 4, 5, 6, 7, and 8 days after emergence (treatments) were released to inside the cage. For each treatment (couple age), 30 replicates were performed. The mating activity of each couple were assessed every 30 min (24 h a day) for 7 days.【Results】Using our laboratory colony, we studied the mating behavior of *D. citri* when individual couples were kept in the laboratory on a common host, *M. exotica*, and under conditions of photoperiod, relative humidity, and temperature mimicking those found under natural conditions in most citrus fields in south China. The results showed that there was a sex pheromone association between the *D. citri* adults. The virgin males were significantly attracted to the virgin females (x^2=6.533, p<0.05) over control. Neither the virgin females nor the mated adults had significant preference for the virgin adults of the opposite sex. There was no mating activity of *D. citri* observed on 1- and 2-days after emergence. The mating behavior of *D. citri* occurred from 3- to 14-days after emergence, and reached a clear peak with 6-day old insects with the highest frequency of copulation (24.4%) and recopulation (79%). During the 3-6 days after emergence, the mating rate gradually increased with the increase of the emergence days, and then gradually decreased at 6-10 days after emergence. The mating rate at 11-days after emergence increased again. Subsequently, the mating rate decreased again at 12-14 days after emergence, but the decline was small. Next, we studied the diel rhythm of mating activity by 6-day-old couples. Mating occurred throughout light period (6:00—20:00) and dark period (20:00—6:00), but mainly in the light period, especially at 14:30—18:30. Within the light period, the mating rate of *D. citri* was low without a regular distribution from 6:00 to 14:00. During the period from 6:00 to 15:30, the mating rate generally increased, and then slightly decreased at 16:00. The mating rate decreased from 16:30 to 20:00. It is obvious that the mating rate in the dark period was lower than 10%. Moreover, no mating activity was observed at 00:30, 01:00, 03:00 and 04:30.【Conclusion】The virgin males were significantly attracted to the virgin females. The mating behavior of *D. citri* occurred until 3 days after emergence. The peak of mating activity occurred at 14:30—18:30 in the afternoon of the 6th day after emergence. It was suggested to extract the sex pheromone of *D. citri* using the 6-day-old couples during this period. To study the mating behavior and pattern of *D. citri* can provide a reliable basis for the extraction, isolation and identification of its sex pheromone components.

Key words: *Diaphorina citri* Kuwayama; Attractive activity; Mating rhythm; Y-tube olfactometer

柑橘木虱(*Diaphorina citri* Kuwayama),属半翅目(Hemiptera)扁木虱科(Liviidae),危害柑橘、月橘、枸橼、黄皮、九里香等芸香科植物,是柑橘的重要害虫,也是柑橘生产上最具毁灭性病害柑橘黄龙病的重要传播媒介,给柑橘生产带来严重威胁[1-2]。近年来,国内外研究者对柑橘木虱行为学的研究表明,雌成虫会释放出有挥发性的性引诱物引诱雄虫前来交配[3],随后又发现柑橘木虱雌成虫或带有雌成虫的寄主植物能引诱雄虫前来取食[4-5]。Mann等[6]研究了柑橘木虱雄成虫和雌成虫的角质层提取物对其行为反应的影响,结果表明雌成虫角质层提取物对雄成虫有引诱作用,而雄成虫角质层提取物没有引

诱作用，并通过进一步分析发现雌雄虫角质层提取物成分存在差异，生物活性试验表明雌成虫角质层提取物碳氢化合物十二烷酸是吸引雄成虫的活性物质。Zanardi等[7]报道柑橘木虱雌成虫发出的气味挥发物醋酸二十四碳酸酯（24Ac）的分解物乙酸能显著引诱雄成虫。上述研究结果表明，柑橘木虱成虫之间存在性信息素的联系。

绝大多数昆虫由雌成虫释放性信息素来唤起雄虫的求偶反应，引诱雄成虫进行交配。雌性昆虫产生的性信息素的量一般仅纳克级（1~50 ng）[8]，因此为了有效分析性信息素成分，了解性信息素释放的高峰期时间非常必要。许多昆虫表现出明显的时间节律，其羽化、交尾和性信息素的产生与释放节律均存在一致性[9-13]。通过对昆虫交配和性信息素释放时间节律的探究，可为信息素有效提取和分离提供可靠依据[14]。笔者在本试验中通过探究柑橘木虱交配规律，确定其交配高峰期，为柑橘木虱性信息素的有效提取和分离提供可靠理论依据。

1　材料和方法

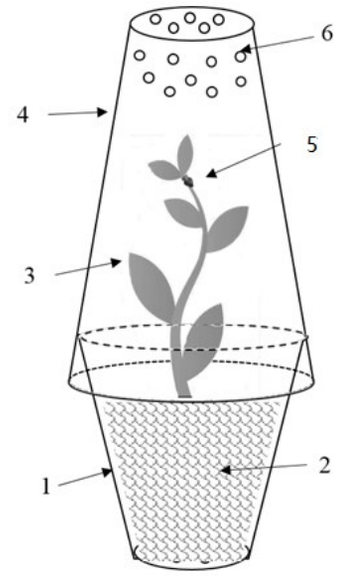
1.1　供试材料

虫源来源于华南农业大学九里香植株上，经室内多代扩繁，雌雄虫配对产卵，孵化直至大龄若虫，挑选龄态、大小一致的大龄若虫用塑料杯罩在九里香苗上单头饲养（图1）。盛培养土的小塑料杯底部直径4.5 cm，开口直径6.5 cm，高8.5 cm，且底部设有排水孔；用作杯罩的大塑料杯底部直径5.5 cm，开口直径8.5 cm，高13.5 cm，且设有透气孔。

九里香苗作为寄主植物，饲养配对及单头的柑橘木虱，观察其交配情况。九里香苗株高8~10 cm，定期进行修剪，当九里香苗长出的嫩梢超过1 cm时，按照雌雄虫1∶1配对接入柑橘木虱成虫2对，保持上述的培养条件任其繁殖。当其发育至4或5龄老熟若虫时，用毛笔把老熟若虫小心地接入装置，1个装置饲养1头若虫，在温度（27±2）℃、湿度60%~70%的条件下继续培养直至羽化。

1.2　试虫处理

单头饲养的柑橘木虱羽化后，测定未交配雄虫对10头处女雌虫、处女雌虫对10头未交配雄虫、已交配雄虫对10头处女雌虫、已交配雌虫对10头未交配雄虫共4种处理的嗅觉行为反应，对照组均不放诱源，为同样大小、形状的空瓶子。



1. 小塑料杯；2. 营养土；3. 九里香苗；4. 大塑料杯；5. 柑橘木虱若虫；6. 透气孔。

1. Small plastic cup; 2. Nrtritional soil; 3. *Murraya exotica* seedling; 4. Big plastic cup; 5. Nymphal *Diaphorina citri*; 6. Air hole.

图1　单头饲养及繁殖柑橘木虱的装置

Fig. 1　The device for rearing and single *Diaphorina citri* in *Murraya exotica*

1.3　柑橘木虱雌雄成虫间引诱活性测定

采用Y型嗅觉仪（郑州谱析科技有限公司）测定柑橘木虱雌雄成虫间相互引诱的活性，根据柑橘木虱虫体大小而设计的Y型嗅觉仪两侧臂长15 cm，内径2 cm；直臂长30 cm，内径3 cm；接虫口内径2.5 cm，两侧臂夹角75°。各装置间用Teflon管联接，其基本组成如下：空气吹气泵→活性炭柱空气过滤装置→空气湿润装置→味源瓶→流量计→Y型管。两侧臂气流控制在200 mL·min^{-1}，试验在温度（27±2）℃、湿度60%~70%的条件下进行。用毛笔将试虫接进Y型管直臂的进虫口处，从试虫进入管口开始计时，反应时间为3 min，当试虫进入侧臂基部1/3处，记录为试虫对该臂气味源做出选择；如果试虫进入直臂5 min后，仍不做出选择，就结束对该试虫的行为观察，不记录结果。每测定5头虫后，调换两侧臂的位置，以消除几何位置对试虫行为产生影响；每测定10头虫后，用无水乙醇清洗Y型管并烘干待用，同时更换另一支干净的Y型管。每次只测试1头虫，每头虫只用1次，每个处理测试30头虫。

1.4　柑橘木虱交配行为的观察

选择羽化后1、2、3、4、5、6、7、8 d未交配的柑橘

木虱成虫作为8个处理，每个处理30对，成对接入九里香苗培养装置中，观察其交配情况。观察环境：温度保持在（27±2）℃，相对湿度60%~70%，光周期14L∶10D，06:00—20:00为光照期，20:00至翌日06:00为黑暗期，黑暗期观察采用红色灯源，连续观察7 d，每隔30 min观察其交尾情况并做记录。

1.5 数据处理

柑橘木虱雌雄成虫间相互引诱活性的显著性分析应用卡平方适合性检验；不同羽化时间的柑橘木虱交配率：交配率/%=同日龄虫交配对数/配对总数；不同羽化时间的柑橘木虱重复交配率：重复交配率/%=同日龄虫重复交配对数/交配总对数；柑橘木虱不同时间段的平均交配率，选取交配率最高时对应龄期的柑橘木虱昼间节律进行研究：平均交配率/%=同一时间段总交配率/对应龄期组数。所有数据采用SPSS 17.0软件进行分析。

2 结果与分析

2.1 柑橘木虱雌成虫对雄成虫的引诱活性测定

分别以10头未交配雌/雄成虫作为诱源，测定未交配/已交配柑橘木虱对异性的趋性反应。结果表明，只有柑橘木虱未交配雄虫对处女雌虫的选择达到了显著水平，处女雌虫及已交配的柑橘木虱成虫对柑橘木虱异性处女成虫的选择均不显著（表1）。

表1 柑橘木虱雌雄成虫之间的引诱活性

Table 1 Attractive activity of virgin or mated adults to the virgin opposite sex

试虫 Insect	诱源 Lure	处理数 *N*	选择虫数 Selected number		χ^2
			处理 Treatment	对照 Control	
未交配♂ Virgin♂	10头处女♀ Ten virgin♀	30	22	8	6.533*
处女♀ Virgin♀	10头未交配♂ Ten virgin♂	30	16	14	0.133ns
已交配♂ Mated♂	10头处女♀ Ten virgin♀	30	14	16	0.133ns
已交配♀ Mated♀	10头未交配♂ Ten virgin♂	30	16	14	0.133ns

注：*表示差异显著（$p < 0.05$）。

Note: Significance levels of χ^2 (chi-square goodness of fit test) indicated by * ($p < 0.05$).

2.2 不同羽化时间的柑橘木虱交配规律

柑橘木虱在羽化后1~2 d均无交配活动发生，羽化后3 d开始出现交尾行为，羽化后6 d的柑橘木虱交配率和重复交配率均最高，分别为24.4%（图2）和79%（图3）。羽化后第3~6天，柑橘木虱随着羽化天数的增加，交配率上升的速度较快，随后交配率逐渐降低；直至羽化后11 d，交配率稍微上升，之后缓慢降低（图2）。羽化后4、6、9 d柑橘木虱重复交配率较高，分别为78%、79%和67%（图3）。

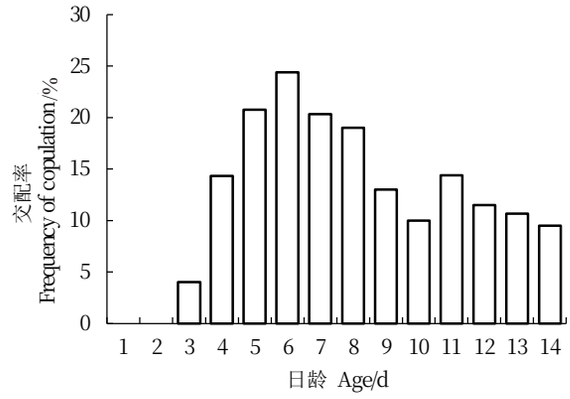


图2 不同日龄柑橘木虱的交配率

Fig. 2 Frequency of copulation of *Diaphorina citri* based on the age

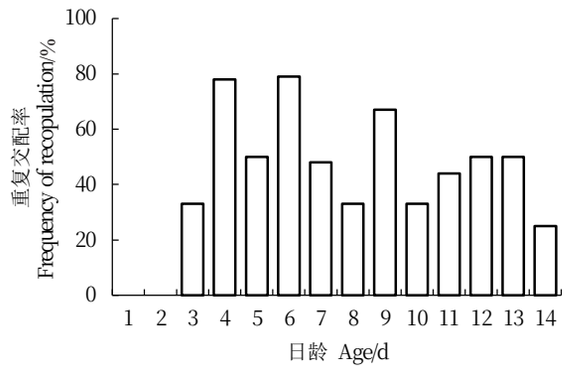


图3 不同日龄柑橘木虱的重复交配率

Fig. 3 Frequency of recopulation of *Diaphorina citri* based on the age

2.3 柑橘木虱不同时间段的交配规律

羽化后6 d的柑橘木虱交配率最高，因此选取6日龄柑橘木虱研究其昼间交配节律，结果表明，柑橘木虱交配活动有一定昼夜规律，光照期（06:00—20:00）的平均交配率要比黑暗期（20:00至翌日06:00）高，占总交配的87.65%，且集中在14:30—20:30时间段，占总交配的65.84%；15:30和16:30时间段的平均交配率最高，均为18%（占总交配的7.41%）（图4）。光照期内，柑橘木虱在06:00—14:00时间段，平均交配率均较低，且呈不规律分布；在光照刚结束1 h（21:00）内，平均交配率为5%~10%，而

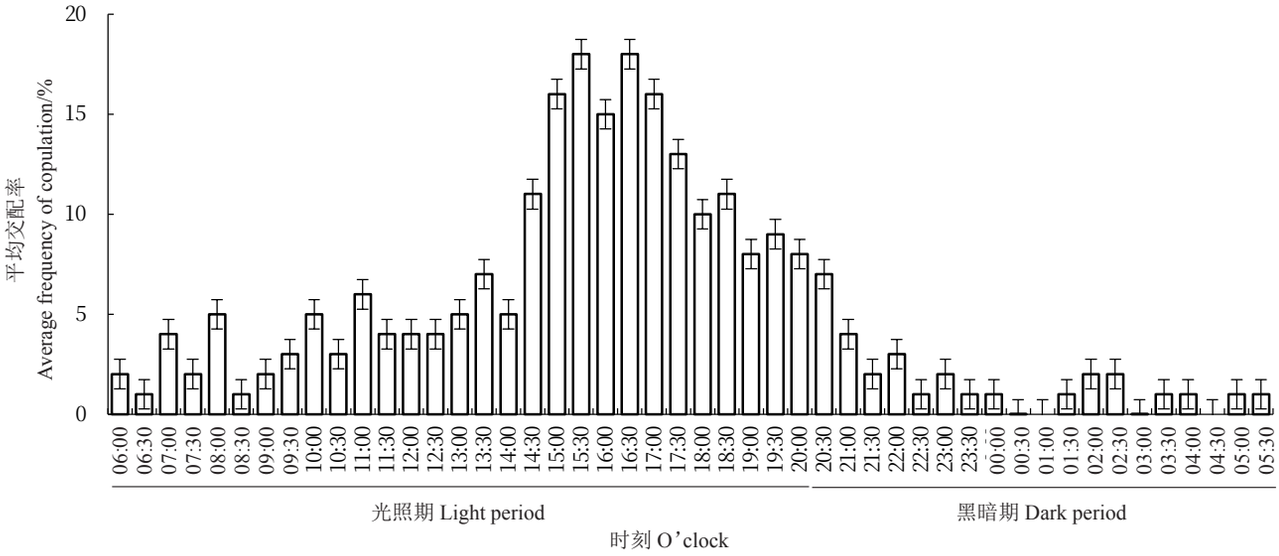


图4　不同时间段柑橘木虱的平均交配率

Fig. 4　Diel rhythm of average frequency copulation of *Diaphorina citri*

在光照结束1 h后，平均交配率很低，均在5%以下，且在00:30、01:00、03:00和04:30时间段未观察到交配现象。柑橘木虱在14:30—18:30平均交配率较高，可在此时间段内提取柑橘木虱的性信息素。

3　讨　论

本研究结果显示，柑橘木虱处女雌虫能显著引诱未交配雄虫，为雌虫产生的挥发性引诱物信息素提供了行为证据。美国实验室观察到的结果为雌性木虱释放的气味并没有增强雌性木虱对交配后雄性木虱的吸引力，这与本研究结果不同，可能是因为试虫的日龄、测试时间以及地理种群的差异[3,15]。

柑橘木虱在成虫羽化后3 d才出现交配行为，且在第6天达到交配高峰，存在重复交配现象，交配日龄及交配高峰期均比Zanardi等[7]的研究早1 d。黎海霖等[16]研究报道柑橘木虱雌雄成虫交配前期分别为10.64和9.84 d，雌雄虫交配比例最大值均为羽化后第9天。这种差异可能是各研究者的试验方法不同导致的，但相同的是柑橘木虱当天羽化的成虫不接受交配，说明柑橘木虱成虫初羽化都需要经历一段时间的取食和性成熟期才能进行交配活动，而这是扁木虱科昆虫繁殖活动特征之一，如中国梨木虱（*Psylla pyrisuga*）亦是如此[17]。柑橘木虱交配高峰期为14:30—18:30时间段，而Wenninger等[18]报道柑橘木虱成虫交配活动主要在白天，但没有明显的交配高峰期。与前人研究[7,16]不同的是，本研究发现柑橘木虱除了00:30、01:00、03:00和04:30时间段没有发生交配行为，其他时间段均有交配活动，这可能是地理种群差异引起的。

4　结　论

柑橘木虱处女雌虫能显著引诱未交配雄虫，柑橘木虱在成虫羽化后3 d才出现交配行为，且在第6天达到交配高峰。柑橘木虱交配活动昼夜节律集中发生在14:30—20:30，交配的高峰期为14:30—18:30。可根据柑橘木虱雌雄成虫的交配高峰期，在14:30—18:30时间段对羽化后6 d的柑橘木虱雌虫进行性信息素的提取。

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