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The effects of non-host plant extracts on the oviposition deterrent and ovicidal activity of *Conopomorpha sinensis* Bradley (Lepidoptera: Gracillariidae)

Qiong Yao[1], Yizhi Dong[1], Wenjing Li[1], and Bingxu Chen[1,]*

Abstract

Conopomorpha sinensis Bradley (Lepidoptera: Gracilariidae) is the most destructive borer pest of litchi, *Litchi chinensis* Sonn., and longan, *Euphoria longan* (Lour.) Steud. (both Sapindaceae) fruits in Southeast Asia and southern China. In this study, ethanol extracts from leaves of *Lantana camara* L. (Verbenaceae), *Wedelia chinensis* (Osbeck) Merr. (Compositae), *Eupatorium odoratum* (L.) King and Robinson (Compositae), *Bauhinia purpurea* L. (Leguminosae), and *Melaleuca leucadendron* L. (Myrtaceae) were chosen to evaluate the oviposition deterrent effect and ovicidal activity of non-host plant extracts on *C. sinensis*. The plant extracts from *L. camara*, *W. chinensis*, and *E. odoratum* caused oviposition deterrent behavior by the adult *C. sinensis*. The ethanol extracts from *L. camara* were found to have the maximum effective deterrence (i.e., 93.5% in the choice test, and 73.6% in the no-choice test). This led to the lowest oviposition activity index (−0.9) and the highest reduction level of egg-laying (3.3 eggs daily per female). In the assessment of ovicidal activity of the plant extracts on *C. sinensis*, *W. chinensis* caused diminished hatching rate (< 60%) at concentrations of 0.01 g·mL^{-1} and 0.1 g·mL^{-1}. These encouraging results suggest that secondary substances from *L. camara* and *W. chinensis* have effective oviposition deterrent and ovicidal activities to *C. sinensis*, and these substances have potential as plant protectants for litchi and longan fruits.

Key Words: fecundity; litchi; longan

Resumen

Conopomorpha sinensis Bradley (Lepidoptera: Gracilariidae) es la plaga barrenadora más destructiva de los frutos de litchi y del ojo de dragón en el sudeste de Asia y el sur de China. En este estudio, escojimos extractos de etanol de hojas de *Lantana camara* L. (Verbenaceae), *Wedelia chinensis* (Osbeck) Merr. (Compositae), *Eupatorium odoratum* (L.) King & Robinson (Compositae), *Bauhinia purpurea* L. (Leguminosae) y *Melaleuca leucadendron* L. (Myrtaceae), para evaluar el efecto disuasorio sobre la oviposición y actividad ovicida de extractos de plantas no hospederas sobre *C. sinensis*. Los extractos de plantas de *L. camara*, *W. chinensis* y *E. odoratum* causaron un comportamiento disuasorio de la oviposición en los adultos de *C. sinensis*. Se descubrió que los extractos de etanol de *L. camara* tienen el mayor efecto de disuasión efectiva (93.5% en la prueba de elección y 73.6% en la prueba de no elección). Esto resultó en el índice más bajo de actividad de oviposición (–0.9) y al mayor nivel de reducción de puesta de huevos (3.3 huevos diarios por hembra). En la evaluación de la actividad ovicida de los extractos de plantas sobre *C. sinensis*, *W. chinensis* causó una disminución de la tasa de eclosión (< 60%) a concentraciones de 0.01 g·mL^{-1} y 0.1 g·mL^{-1}. Estos resultados prometores sugieren que las sustancias secundarias de *L. camara* y *W. chinensis* tienen actividades efectivas de prevención de oviposición y ovicidas para *C. sinensis*, y que estas sustancias tienen un potencial como protectores de plantas para las frutas del litchi y del ojo de dragón.

Palabras clave: fecundidad; litchi; ojo de dragón

Litchi, *Litchi chinensis* Sonn., and longan, *Euphoria longan* (Lour.) Steud. (both Sapindaceae), are native to southern China, and are 2 of the most dominant and valuable fruits in India, Nepal, Taiwan, Thailand, Vietnam, and China. The cultivated area and fruit yield of these 2 fruits in China are the highest in the world (Houbin et al. 2013). *Conopomorpha sinensis* Bradley (Lepidoptera: Gracilariidae) is the most destructive borer pest specifically attacking litchi and longan, causing significant economic loss in southeastern Asia and southern China. *Conopomorpha sinensis* lays eggs on litchi and longan fruit any time after flowering; the larvae penetrate the fruits immediately after hatching, feed on the seed stalk, and spend the entire larval stage inside the seed (Menzel 2002; Pengyan et al. 2014). Thus, control of the *C. sinensis* larvae is hampered because of its life habits, specifically the time and zone restrictions of its occurrence. Though chemical control of *C. sinensis* adults is effective, chemical insecticides have produced undesirable effects, such as toxicity to non-target organisms (including a large number of beneficial natural enemies), fostered environmental and human health concerns, and induced resistance to synthetic chemical insecticides (Bingxu et al. 2011). Environmentally preferable pest control strategies should be developed for this borer pest control.

Plants can produce a variety of secondary compounds with insecticidal and antimicrobial effects, including alkaloids, flavonoids, steroids, phenols, terpenoids, unique amino acids, and polysaccharides (Roy et al. 2016; Velasques et al. 2017). Extensive research

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has revealed that the secondary metabolites and defensive proteins of plants can have different impacts on insects, including attracting, repelling, and even killing the insects, and inhibiting growth of the insect. For example, volatile oils of *Cymbopogon citrates* (DC.) (Poaceae) and *Eugenia uniflora* L. (Myrtaceae) can reduce the adult emergence of *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae) and protect cowpea from weevil attack (Gbolade & Adebayo 1993). Essential oils extracts from leaves of *Ageratum conyzoides* L. (Asteraceae), *Lantana camara* L. (Verbenaceae), and *Chromolaena odorata* (L.) (Asteraceae) could cause significant mortality of *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in 24 h (Bouda et al. 2001). The leaves of *Parthenum hysterophorus* could cause over 70% deterrence in the oviposition behavior of *Aedes aegypti* (L.) (Diptera: Culicidae) adults (Kumar et al. 2011). A crude extract of *Artemisia annua* L. (Asteraceae) had larvicidal and ovicidal effects for *Culex quinquefasciatus* Say (Diptera: Culicidae) and caused more than 85% oviposition deterrence compared with the control group (Cheah et al. 2013). *Cabralea canjerana* (Vell.) (Meliaceae) extracts diminished pupal viability and highly hampered oviposition of *Anastrepha fraterculus* (Weidemann) (Diptera: Tephritidae) (Magrini et al. 2014). Given the limited strategies currently available for control of *C. sinensis* during the larval and egg stages, overuse of pesticides, and difficulty of introduction of natural enemies, studies of essential oils and plant extracts from plants were undertaken as possible alternative management tactics in litchi and longan orchards. *Lantana camara* L. (Verbenaceae), *Wedelia chinensis* (Osbeck) Merr. (Compositae), *Eupatorium odoratum* (L.) King and Robinson (Compositae), *Bauhinia purpurea* Linn. (Leguminosae), and *Melaleuca leucadendron* Linn. (Myrtaceae) are 5 plants that occur widely in southern China. The purpose of this study was to evaluate these 5 non-host plant extracts for control of *C. sinensis*. Specifically, we investigated the 5 plant extracts for attraction or repellence, and their effects on fecundity of *C. sinensis* adults. We also determined the ovicidal activity of these 5 plants' extracts against *C. sinensis*.

Materials and Methods

PLANT COLLECTION AND PREPARATION OF PLANT EXTRACTS

The leaves of *L. camara*, *W. chinensis*, *E. odoratum*, *B. purpurea*, and *M. leucadendron* were collected from the South China Agricultural University campus, Guangdong Academy of Agricultural Sciences and Guangzhou Baiyun District Zhongluotang Agricultural Base, Guangzhou, People's Republic of China.

The leaves were washed thoroughly with distilled water 3 times, then dried for 7 d in the shade at a room temperature of 27 to 32 °C. The dried leaves were powdered using a mechanical grinder. Fifty grams of powdered leaves were soaked in 300 mL of 95% ethanol, and were left in the shade at room temperature for 24 h. The extracts from the plant leaves were concentrated in a rotary vacuum evaporator, and the dark greenish residues obtained were stored in a −80 °C refrigerator until use. One g of each crude plant extract was diluted with 1 mL of 95% ethanol, and used at the concentration of 0.1 g·mL^{-1} in the oviposition deterrence bioassay and fecundity study, and 0.1 g·mL^{-1} and 0.01 g·mL^{-1} concentrations were used in the ovicidal bioassay (Jidong et al. 2001; Rehman et al. 2009).

INSECTS

Falling litchi fruits were collected at various litchi and longan orchards in Guangzhou in the northeast of Guangdong Province of China. *Conopomorpha sinensis* pupae were collected from the fallen fruits. Adult moths were raised in constant-temperature incubators at 26 ± 1 °C and 65 to 85% relative humidity (RH) with a 14:10 h (L:D) photoperiod ratio using 20% (v/v) diluted honey as a food source (Qiong et al. 2016).

Fresh litchi fruits (cv. 'Nuomici') were collected in a litchi and longan orchard in Guangzhou Baiyun District Zhongluotang Agricultural Base, Guangzhou, People's Republic of China. They were washed thoroughly with distilled water and used as oviposition stimulants in the oviposition bioassay.

OVIPOSITION DETERRENCE ASSAY

Egg-laying paper was prepared as follows: 1 layer of tissue paper was soaked in 95% ethanol for 2 sec, and dried thoroughly at room temperature before use. Clean fresh litchi fruits were covered with egg-laying paper that had been soaked in plant extracts at a concentration of 0.1 g·mL^{-1} for 10 sec. They then were dried in the laboratory for 1 h and used on treated fruit for adult oviposition substrates in each treatment. Litchi fruits soaked in 95% ethanol were used as untreated fruit in the control group. Five-d-old females and males of *C. sinensis* were chosen arbitrarily and used in the oviposition deterrence assay. Ten males and 10 females were placed in each insect rearing cage (with dimensions of 30 × 30 × 30 cm). Each cage was considered a replicate and there were 3 replicates per treatment.

Both choice and no-choice tests were used to investigate the attractant or repellent effects of plant extracts on *C. sinensis* adults. In the choice test, 4 treated and 4 untreated fruits were randomly arranged in an insect rearing cage. In the no-choice test, 10 treated fruits or 10 untreated fruits were placed separately in 2 different insect rearing cages. The oviposition activity indexes were assessed 24 h after the beginning of the experiment.

The percent effective deterrence (ED) for each kind of plant extract in both the choice test and no-choice test were calculated using the following formula (Elango et al. 2011):

$$ED = \frac{Nc - Nt}{Nc} \times 100$$

where Nc is the number of eggs in the control group and Nt is the number of eggs in the treated group.

The oviposition activity indexes (OAIs) for each plant extract in the choice test was calculated using the following formula (Kramer & Mulla 1979):

$$OAI = \frac{Nt - Nc}{Nt + Nc}$$

FECUNDITY STUDIES

One-d-old females and males of *C. sinensis* were selected randomly to investigate the effects of different plant extracts on the fecundity and fertility of *C. sinensis*. Six pairs of *C. sinensis* were placed in each insect rearing cage. Each cage was considered as a replicate and each treatment had 3 replicated cages. The replacement of oviposition substrate (i.e., fresh litchi fruits wrapped with treated egg-laying paper) and the collection of eggs were carried out every 24 h for 5 d. For each extract, relative fecundity was calculated by dividing the total number of eggs collected in each treatment group by the total number of eggs collected in the control group (Kumar et al. 2011).

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OVICIDAL ASSAY

To investigate the ovicidal activity of plant extracts on the eggs of *C. sinensis*, the egg-laying paper was replaced daily and the freshly laid eggs were collected. The eggs were submerged in the aforementioned 2 different concentrations of plant extracts for 10 sec. Eggs from each replicate were kept separate for hatching assessment. The hatched eggs were counted daily until no eggs hatched for at least 48 h.

Percent hatchability of eggs was calculated using the following formula:

$$\text{Hatchability} = \frac{\text{Number of larvae hatched}}{\text{Total number of eggs in each replicate}} \times 100$$

STATISTICAL ANALYSIS

The experimental data was analyzed using the 1-way analysis of variance (ANOVA). Means were separated by Tukey's test in SPSS 18.0. All levels of statistical significance were determined at $P < 0.05$.

Results

OVIPOSITION REPELLENT EFFECT OF DIFFERENT PLANT EXTRACTS ON *CONOPOMORPHA SINENSIS*

The effects of the 5 plant extracts for control of *C. sinensis* adults are presented in Table 1. Ethanol extracts from *L. camara*, and to a lesser degree *W. chinensis* and *E. odoratum*, had promising oviposition repellent effects on *C. sinensis* adults in the choice and no-choice tests. In both tests, the use of ethanol extracts from *L. camara* resulted in the highest effective deterrence (93.5% in choice test, and 73.6% in no-choice test), which was significantly greater than the other extracts. In the choice test, leaf extract from *W. chinensis* and *E. odoratum* induced effective deterrence of 61.4% and 54.2%, respectively, to oviposition of *C. sinensis*. In contrast, in the no-choice test the effective deterrence values of ethanol extracts from these 2 plants were only 25.3% and 15.6%, respectively.

The oviposition activity index values of *L. camara*, *W. chinensis*, and *E. odoratum* were all below −0.3. This indicates that the ethanol extracts from these 3 plants could be considered as oviposition repellents for *C. sinensis* adults (Fig. 1). Additionally, *L. camara* was found to be the most effective plant in repelling the oviposition behavior of *C. sinensis* adults, leading to the lowest oviposition activity index value (−0.9) and the highest reduction in egg-laying on each litchi fruit.

EFFECT OF PLANT EXTRACTS ON THE FECUNDITY OF ADULT *CONOPOMORPHA SINENSIS*

The leaf extracts from these 5 non-host plants affected the fecundity of *C. sinensis* adult females. *Conopomorpha sinensis* adult females laid significantly fewer eggs on litchi fruits after treatment with *L. camara* extracts compared to the other 4 plant extracts. The *L. camara* extracts caused the most diminished relative fecundity (0.3) (Table 2). Daily egg production per female treated with leaf extracts of *W. chinensis* and *E. odoratum* was reduced to 6.3 and 9.6, respectively. However, the daily egg production per female after treatment with *B. purpurea* and *M. leucadendron* extracts was not affected as compared to the control group, which means that the leaf extracts from these 2 plants exhibited no significant effects on fecundity of *C. sinensis* adult females.

OVICIDAL ACTIVITY OF DIFFERENT PLANT EXTRACTS TO THE EGGS OF *CONOPOMORPHA SINENSIS*

Conopomorpha sinensis egg hatchability after treatment with the ethanol leaf extracts at concentrations of 0.1 $g \cdot mL^{-1}$ and 0.01 $g \cdot mL^{-1}$ are presented in Table 3. The highest egg mortality was observed in the treatment group of ethanol extracts from *W. chinensis*, followed by extracts from *L. camara*. The ethanol extracts from *W. chinensis* caused the most diminished hatching rates (< 60%) among the 5 plant extract treatments. *Lantana camara* leaf extracts at a concentration of 0.1 $g \cdot mL^{-1}$ reduced the egg hatchability to 71.7 ± 2.3%, but *L. camara* extracts with a concentration of 0.01 $g \cdot mL^{-1}$ had no significant influence on egg hatching compared to the control group. The egg hatchability after treatment with 2 concentrations of *B. purpurea*, *E. odoratum*, and *M. leucadendron* leaf extracts had no significant effects as compared to the control group (Table 3).

Discussion

In the evolution of insects and plants for hundreds of millions of years, plants have gradually produced defensive secondary compounds that are protective, repellent, and toxic to insects (Isman 2006; Züst & Agrawal 2016). Botanical extracts from the plant seeds, flowers, leaves, and roots have been used to protect the plant from pests and affect the oviposition behavior of insects (Diaz 2016; Soujanya et al. 2016). This has effectively controlled the population dynamics of pests on the plant for centuries. In fact, many studies have been carried out to reveal the effectiveness of plant extracts or essential oils to repel mosquitos (Maia & Moore 2011; Rehman et al. 2014). However, the studies on effectiveness of botanical extracts against agricultural pests are less common.

Table 1. The oviposition deterrent effect of different plant extracts on *Conopomorpha sinensis*.

	Choice test			No-choice test	
Plant	Egg number per treated fruit	Egg number in control per fruit	ED (%)	Egg number per treated fruit	ED (%)
L. camara	1.8 ± 0.6	28.0 ± 1.5	93.5	4.8 ± 1.1 d	73.6
W. chinensis	13.0 ± 0.8	33.7 ± 2.0	61.4	18.9 ± 1.2 c	25.3
E. odoratum	19.2 ± 1.6	41.8 ± 1.9	54.2	23.2 ± 1.8 bc	15.6
B. purpurea	30.7 ± 1.2	38.7 ± 1.5	20.7	31.4 ± 1.6 a	0.5
M. leucadendron	36.5 ± 2.6	35.7 ± 1.6	−2.3	30.1 ± 1.5 ab	2.7
Control	–	–	–	31.8 ± 1.5 a	–

The data in the table are mean ± SE, and means in a column followed by different letters indicated significant differences (Tukey's test: $P < 0.05$). ED = effective deterrence.

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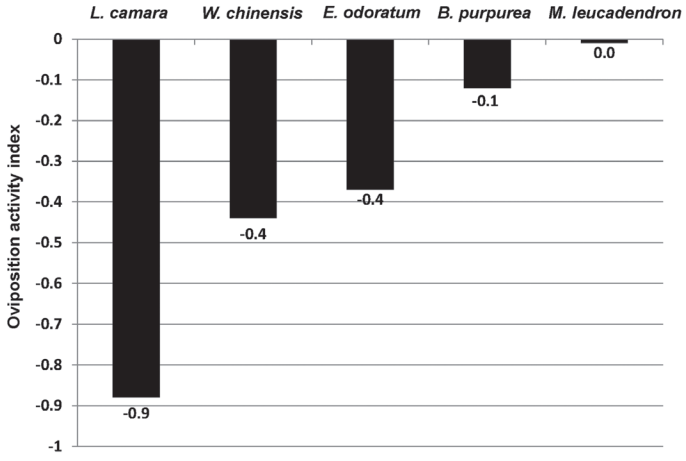


Fig. 1. The effect of 5 plant extracts on oviposition activity index (OAI) values of *Conopomorpha sinensis* in the choice test. Plant extracts with oviposition activity indexes of ≥ 0.3 are considered as attractants, whereas those of ≤ −0.3 are considered to be deterrents. Negative values indicate that more eggs were deposited in the control group than those in groups receiving treatment with plant extracts.

Changlong et al. (2001) reported that the chloroform extracts from the bark of *L. camara* showed 32.4% oviposition deterrence in choice tests with *C. sinesis*, and 43.0% oviposition deterrence in no-choice tests at a concentration of 0.1 g·mL^{-1}. Jidong et al. (2001) and Xiaoqin et al. (2008) reported that spraying both ethanol extracts and chloroform extracts from the bark of *E. odoratum* could deter *C. sinensis* adults from laying eggs in a litchi orchard. Thus, extracts from the plants we studied have demonstrated activity previously on oviposition of *C. sinesis*. However, their ovicidal activity and their effects on *C. sinensis* fecundity are unclear. In our study, the oviposition deterrent effect and ovicidal activity of extracts from *L. camara*, *W. chinensis*, *E. odoratum*, *B. purpurea*, and *M. leucadendron* were evaluated on *C. sinensis*. The deterrent activities of *L. camara* and *E. odoratum* on *C. sinensis* were confirmed by using ethanol leaf extracts of these 2 plants, but were much higher than the deterrent activities of chloroform bark extracts against *C. sinensis* reported in previous studies. When given a choice, the *C. sinensis* avoided fruits treated with leaf extracts of *L. camara*, *W. chinensis*, *E. odoratum*, and *B. purpurea*. When no choice was given, *C. sinensis* adults also would land on treated fruits but laid fewer eggs. This indicates that these 3 plants contain a substance for *C. sinensis* oviposition deterrence. Moreover, the lowest oviposition activity index value (−0.9) and relative fecundity (0.3) of *C. sinensis* were observed after treatment with *L. camara* extracts. Thus, these plant leaf extracts can be considered the most effective oviposition deterrents in our study, although ethanol leaf extracts from *W. chinensis* and *E. odoratum* also affected the fecundity of *C. sinensis* adult females. The *L. camara* extracts diminished the hatchability at a concentration of 0.1 g·mL^{-1}, whereas no significant difference of hatching rate was observed at a concentration of 0.01 g·mL^{-1}. The results indicate that *C. sinensis* eggs were tolerant to leaf extracts from *L. camara*. The lowest hatching rate (< 60%) of *C. sinensis* eggs were observed after treatment with *W. chinensis* extracts at 2 concentrations. This indicates that *W. chinensis* may contain an ovicidal substance to control *C. sinensis*. This is the first report of ovicidal activities of botanical extracts on *C. sinensis*.

This investigation clearly demonstrated the effects of 5 non-host plant leaf extracts on *C. sinensis*, the most destructive borer pest of litchi and longan. These results indicate that the secondary substances from 3 non-host plants, namely *L. camara*, *W. chinensis*, and *E. odoratum*, are effective oviposition deterrents on adult *C. sinensis*, and could cause reduction in fecundity of *C. sinensis*. Moreover, the second substances from *W. chinensis* have ovicidal effects on *C. sinensis* eggs. These data indicate potential for extracts from *L. camara* as a repellent for oviposition, and extracts from *W. chinensis* as a botanical ovicide of *C. sinensis*. However, there is a concern that the application of extracts from *L. camara* and *W. chinensis* as plant protectants may disrupt the occurrence of natural enemies and pollinating insects. Field studies on the impacts of these 2 plants against other insects are needed. New insights on green plant protectants provide the potential for more environmentally preferable strategies for pest management.

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Table 2. The effect of different plant extracts on the fecundity of adult *Conopomorpha sinensis*.

Plant	No. of females (total females)	Total eggs	Daily eggs per female	Relative fecundity
L. camara	6 (18)	98.30s ± 5.0 (295)	3.30 ± 0.2 d	0.25
W. chinensis	6 (18)	190.30 ± 17.9 (571)	6.30 ± 0.6 c	0.48
E. odoratum	6 (18)	289.30 ± 22.7 (868)	9.64 ± 0.6 b	0.73
B. variegata	6 (18)	353.70 ± 19.1 (1061)	11.80 ± 1.1 ab	0.89
M. leucadendron	6 (18)	386.00 ± 17.0 (1158)	12.90 ± 0.6 a	0.98
Control	6 (18)	395.70 ± 19.0 (1187)	13.20 ± 0.6 a	—

Relative fecundity was calculated by dividing the total numbers of eggs collected in each treatment group by the total number of eggs collected in the control group. The data in the table are mean ± SE, and means in a column followed by different letters indicate significant differences (Tukey's test: $P < 0.05$).

Table 3. The hatchability of *Conopomorpha sinensis* after treatment with different plant extracts.

	Percentage of egg hatching (mean ± SE)					
Concentration (g·mL^{-1})	*L. camara*	*W. chinensis*	*E. odoratum*	*B. variegata*	*M. leucadendron*	Control
0.1	71.7 ± 2.3 b	54.8 ± 2.4 c	89.8 ± 0.7 a	89.0 ± 0.7 a	87.9 ± 2.7 a	89.8 ± 2.8 a
0.01	85.4 ± 2.3 a	59.7 ± 2.3 b	91.8 ± 1.3 a	89.4 ± 2.8 a	89.1 ± 2.2 a	91.8 ± 0.4 a

The data in the table are mean ± SE, and means in a column followed by different letters indicate significant differences (Tukey's test: $P < 0.05$).

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