Evaluation of the effectiveness of insecticide trunk injections for control of *Latoia lepida* (Cramer) in the sweet olive tree *Osmanthus fragrans*

Jun Huang [1], **Juan Zhang** [1], **Yan Li** [2], **Jun Li** [Corresp., 3], **Xiao-hua S Shi** [1]

[1] Flower Research and Development Centre, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang Province, China

[2] The Key Laboratory of Chemistry for Natural Products of the Guizhou Province, Chinese Academy of Sciences, Guizhou, Guiyang Province, China

[3] Guangdong Key Laboratory of IPM in Agriculture and Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Institute of Applied Biological Resources, Guangzhou, Guangdong Province, China

Corresponding Author: Jun Li
Email address: junlee100@163.com

The screening of suitable insecticides is a key factor in successfully applying trunk injection technology to ornamental plants. In this study, six chemical pesticides were selected and injected in to the trunks of *Osmanthus fragrans* to control the nettle caterpillar, *Latoia lepida* (Lepidoptera: Limacodidae), using a no-pressure injection system. The absorption rate of the insecticides, the leaf loss, and the mortality and frass amount of *L. lepida* larvae were evaluated after 77 and 429 d ays. The results showed that 4% imidacloprid + carbosulfan and 21% abamectin + imidacloprid + omethoate had the fastest conductivity and were completely absorbed into the trunks within 14 days; however, the efficiencies of these insecticides were extremely low. Additionally, 10% emamectin benzoate + clothianidin and 2.5% emamectin benzoate were almost completely absorbed within 30 days and exhibited a longer duration of insecticide efficiency (>80% mortality) in the upper and lower leaves of the canopy. Treatment with these insecticides also resulted in significantly lower leaf loss and frass amounts. We conclude that emamectin benzoate and emamectin benzoate + clothianidin have a rapid uptake into *O. fragrans*, an appropriate efficiency and are effective as insecticides over long durations. Hence, they may be a suitable control option for *L. lepida* in *O. fragrans* plants.

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Jun Huang[1], Juan Zhang[1], Yan Li[3], Jun Li[2*], Xiao-Hua Shi[1]

[1] Flower Research and Development Centre, Zhejiang Academy of Agricultural Sciences, Hangzhou 311202, Zhejiang Province, P.R. China.

[2] Guangdong Key Laboratory of IPM in Agriculture and Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Institute of Applied Biological Resources, Guangzhou 510260, Guangdong Province, P.R. China.

[3] The Key Laboratory of Chemistry for Natural Products of the Guizhou Province and the Chinese Academy of Sciences, Guiyang 550002, Guizhou Province, P.R. China.

Juan Zhang contributed equally with Jun Huang and is a co-first author of this paper.

Corresponding author: junlee100@163.com (Li Jun)

ABSTRACT

The screening of suitable insecticides is a key factor in successfully applying trunk injection technology to ornamental plants. In this study, six chemical pesticides were selected and injected into the trunks of *Osmanthus fragrans* to control the nettle caterpillar, *Latoia lepida* (Lepidoptera: Limacodidae), using a no-pressure injection system. The absorption rate of the insecticides, the leaf loss, and the mortality and frass amount of *L. lepida* larvae were evaluated after 77 and 429 days. The results showed that 4% imidacloprid + carbosulfan and 21% abamectin + imidacloprid + omethoate had the fastest conductivity and were completely absorbed into the trunks within 14 days; however, the efficiencies of these insecticides were extremely low. Additionally, 10% emamectin benzoate + clothianidin and 2.5% emamectin benzoate were almost completely absorbed within 30 days and exhibited a longer duration of insecticide efficiency (>80% mortality) in the upper and lower leaves of the canopy. Treatment with these insecticides also

resulted in significantly lower leaf loss and frass amounts. We conclude that emamectin benzoate and emamectin benzoate + clothianidin have a rapid uptake into *O. fragrans*, an appropriate efficiency and are effective as insecticides over long durations. Hence, they may be a suitable control option for *L. lepida* in *O. fragrans* plants.

INTRODUCTION

The sweet olive, *Osmanthus fragrans* (Thunb.) Lour., is a popular garden evergreen shrub or small tree that belongs to the family of Oleaceae. It has both ornamental and practical uses in landscaping and as incense (*Liu & Xiang, 2003*; *Lee et al., 2007*) and is widely planted in the Huaihe River basin and southern areas of China (*Wang et al.,* 2006). The nettle caterpillar or blue-striped nettle grub, *Latoia lepida* (Cramer; Lepidoptera: Limacodidae), is distributed throughout Southeast Asia (*Azharul Islam et al.,* 2009), especially in China, Japan, India, Sri Lanka, Indonesia and Vietnam (*Hirashima, 1898*). *L. lepida* larvae mainly feed on *O. fragrans* leaves, resulting in restricted growth and dieback of leaves and twigs (*Wakamura et al., 2007*). Thus, this pest reduces the ornamental and practical values of the plants (*Ju et al., 2007*). In addition, exposure to the stinging spines on the dorsal surface of *L. lepida* can cause skin problems in humans such as redness, swelling, heating, and pain as well as other clinical manifestations such as fever, joint pain, and even death in allergic populations (*Qin et al., 1998*). Therefore, combating *L. lepida* infestations is both economically valuable and significant for protecting human health.

Currently, spraying chemicals on tree crowns is the main control method for *L. lepida* in China. However, chemical spraying can release pesticides into the air and water and affect non-target animals, causing adverse consequences such as the deaths of large numbers of natural enemies, livestock poisoning and environmental pollution (*Wakamura et al., 2007*). Chemical spraying is most commonly used in the green areas of cities or in the suburbs. In contrast, trunk injection technology is a more environmentally friendly method of applying pesticides because it is highly efficient for liquid drugs, can be used with a broad spectrum of insecticides and is relatively pollution free, safe, simple to apply, and is barely affected by weather (*Navarro, 1992*; *Montecchio, 2013*). Trunk injection technology involves the injection of pesticides directly into tree trunks, which then transport the liquids through their conductive tissues to the site of action (*Mendel, 1998*; *Harrell, 2006*; *Mota-Sanchez et al., 2009*; *Doccola et al., 2011*); thus, trunk injection can play an important role in disease or insect pest control (*Mota-Sanchez et al., 2009*; *Takai et al., 2001*; *James et al., 2006*; *Darrieutort & Lecomte, 2007*). For example, using trunk injections of emamectin benzoate, ash trees with heavy infestations of *Agrilus planipennis* exhibited less canopy decline over a four-year period compared to non-treated control treesAgrilus planipennis (*Flower et al., 2015*) and also resulted in a nearly 99% mortality of the *A. planipennis* feeding on the treated tissues (*Smitley et al., 2010*; *McCullough et al., 2011*; *Herms*

112 *et al., 2014*).

113 Certainly, having a variety of insecticide options is a key factor in the successful application of
114 trunk injection technology. *Byrne et al.* (2012) found that the uptake of 10% dinotefuran was
115 more rapid than the uptake of 5% imidacloprid in California avocado groves. Both chemicals
116 showed good control of the avocado thrips *Scirtothrips perseae*, and no residues were detected
117 within the fruits. In contrast, although 10% acephate showed a rapid uptake and provided good
118 control of thrips in bioassays, acephate residues and its insecticidal metabolite methamidophos
119 were detected in fruits for up to 4 weeks after the injection. However, the uptake of 5%
120 avermectin was slow, and it was ineffective against avocado thrips (*Byrne et al., 2012*). Another
121 study found that trunk injections of imidacloprid, thiamethoxam and clothianidin in fully grown
122 king mandarin trees to control the citrus greening disease *Diaphorina citri* resulted in
123 approximately 50% mortality of the psyllids within 45 days. In general, imidacloprid had a better
124 control effect than the other insecticides tested (*Ichinose et al., 2010*). Therefore, evaluations of
125 pest control using trunk injections of different chemicals provide a quick and effective
126 assessment of the optimal trunk injection agent. However, little has been reported on the success
127 of insecticide treatments using trunk injection techniques to control *L. lepida* on *O. fragrans*
128 trees.

129 In this study, we selected six chemical pesticides to be injected, without pressure, into the
130 trunks of *O. fragrans* to control *L. lepida*. First, the absorption rates of the insecticides were
131 estimated on different observation days within a month after the trunk-injection application. In
132 addition, the mortality of *L. lepida* larvae and tree leaf loss were evaluated in bioassays to
133 determine the duration of efficacy at 77 (approximately the period between two successive
134 generations of *L. lepida* in a year in China) (*Ju et al., 2007*) and 429 days after treatment. Finally,
135 we also investigated the amount of frass deposited by *L. lepida* larvae at these two time points.
136 Our goal was to assess which type of insecticide performed best with regard to the uptake rate,
137 efficacy against the target pest and effective duration.

138

139 ## MATERIALS AND METHODS

140 ### Plants and insects

141 This study was conducted in a garden located in the Zhejiang Academy of Agricultural Sciences
142 (30°18′75″ N; 120°28′60″ E), Hangzhou, China. The sweet olive trees used in this study, *O.*
143 *fragrans* var. thunbergii, were 10–15 years old and planted in a total of three rows spaced

144 approximately 3 m apart. The trees had well-structured crowns and a uniform growth trend. We
145 randomly selected 21 individual trees and measured their heights, canopy widths, and diameter at
146 chest height, resulting in means (± sem) of 4.53 ± 0.20 m, 2.39 ± 0.10 m, and 0.12 ± 0.01 m,
147 respectively. These trees were managed with common watering and fertilization techniques;
148 however, they were not subjected to chemical pesticides. Fifth instar larvae of *L. lepida* with
149 similar weights were collected from sweet olive trees planted in the Hangzhou Blue Ocean
150 Ecology Park (30°08′71″ N; 120°31′49″ E) and used for the bioassay. None of the study species
151 are protected in China; therefore, no specific permits were required for collections or field
152 activities.

153

154 **Insecticides**

155 The insecticides used in this study included 95% imidacloprid and 70% emamectin benzoate
156 (Guangdong Dafeng Plant Protection Technology Co., Ltd.), 95% abamectin (Hebei Weiyuan
157 Group Co., Ltd.), 95% clothianidin emulsifiable concentrate (Nanjing Lebang Chemical Products
158 Co., Ltd.), 98% omethoate (Lianyungang Dongjin Chemical Co., Ltd.), and 92% carbosulfan
159 (Jiangsu Xingnong Co., Ltd.). These insecticides were diluted and formulated (or mixed)
160 following the six trunk injection chemicals described in Table 1.

161

162 **Insecticide application by trunk injection**

163 On 28 April 2014, 21 brown plastic bottles (Guangdong Institute of Applied Biological
164 Resources supplied, designed by Dr. Li Jun) 6 cm high (from the bottom to the bottle neck) and 4
165 cm in diameter were prepared in the laboratory. Figure 1 shows an outline of the bottle. Each
166 bottle was supplied with 30 mL of insecticide for trunk injection (n = 3 for each treatment).
167 Three bottles were filled with distilled water (no insecticides) as controls. A hole approximately
168 30 mm in depth and 4 mm in diameter was drilled downward in the main trunk of each tree at a
169 45° angle approximately 30 cm above the ground using a rechargeable drill (Model TSR/1080-LI,
170 Bosch Power Tools Co., Ltd., Shanghai, China). The bottle tip was cut open using a razor and
171 inserted into the hole to completely inject the insecticides into the trunk. The screw threads on
172 the tip of the bottle provide a good seal between the bottle and the edges of the drilled hole to
173 prevent chemical leakage. Finally, an approximately 1 mm diameter air hole was made by
174 puncturing the bottom of the bottle with an insect needle (approximately 0.75 mm in diameter
175 and 40 mm in length) to promote the uptake of the insecticides. The quantity of residual agent in

the bottles was visually observed and recorded at 9, 14, 23, and 30 days after application to test whether the absorption rates of the trunk-injected insecticides varied. During the assays, the temperature was 23.40 ± 0.71°C and the atmospheric humidity was 67.75 ± 2.10%; there were 5 rainy days (showers).

Laboratory bioassay

Treated tree branches were sampled at 77 days and 429 days after insecticide application and were brought to a laboratory to test the efficacy of insecticides on the targeted *L. lepida* larvae. Two branches from the bottom and top of the canopy were randomly collected from each tree in any compass direction. Each branch was 25–30 cm in length and had approximately 16 leaves. Debris and insects were removed from the branches and leaves before the test. A similar leaf-residue method (*Busvine, 1980*) and a custom setup were used for the larval bioassay; Figure 2 shows a diagram of its configuration. The specific operational steps were as follows: 1) Each branch was placed vertically in a glass bottle (6 cm in diameter, 9 cm in height) filled with distilled water and sealed at the bottle neck with polystyrene foam; 2) the glass bottles were placed in the center of a plastic funnel (upper diameter, 40 cm; lower diameter, 5 cm; and height, 20 cm); and 3) the funnel was placed on the mouth of another glass bottle with the funnel neck (ca. 4 cm in length) inserted into the glass bottle and secured. This setup served to collect the larval frass in the bottom bottle. The inner wall of the funnel was coated with Teflon cream (Fluon®) to avoid the escape of fallen larvae.

L. lepida larvae (after being starved for 24 h) were transferred to the leaves using a brush and allowed to stabilize for 12 h of observation before the test began. Two larvae were placed on each branch and allowed to feed on leaves for 5 consecutive days during which their frass was collected. The mortality of the larvae feeding on these treated leaves was recorded after 5 days. The efficacy of the insecticide was evaluated based on the recorded mortality. Supplementary evaluation information was also recorded, such as the number of leaves eaten or damaged by larvae, and larval frass was weighed using an electronic scale (model EX223, Ohaus Inc., USA).

Statistical Analyses

Shapiro-Wilks tests were applied to determine whether the data had a normal distribution and homogeneity of variance. When the data were normally distributed and exhibited similar variances, they were further analyzed using a repeated-measures ANOVA to compare the

208 absorption rates between insecticides (between-subject) and between the examination days
209 (within-subject). The mortality of larvae feeding on the upper and lower isolated branches
210 following trunk injection with different insecticides at 77 days or 429 days was analyzed and
211 compared using a two-way ANOVA and Duncan multiple range tests. The same methods were
212 used to compare the differences in the percentages of damaged leaves and frass amounts between
213 insecticides and leaf position (i.e., upper or lower branches). When necessary, data were
214 normalized by either square root or logarithmic transformations. All the statistical analyses were
215 conducted using SPSS 14.0 (SPSS Inc., Chicago, IL, USA).

216

217 ## RESULTS

218 ### Absorption rates of the insecticides

219 The quantities of the six insecticides were reduced according to the number of days that had
220 elapsed after the applications (Fig. 3). In particular, insecticide quantities decreased
221 conspicuously between the 14th and 30th days. The tests showed that the absorption rates of the
222 insecticides differed significantly between insecticides ($F_{5,36} = 8.899$, $P < 0.001$) and observation
223 times ($F_{3,36} = 14.568$, $P < 0.001$), but the interaction between these two factors was not
224 significant ($F_{15,36} = 0.825$, $P = 0.686$). Within 30 days of the injection, 4 of the insecticides were
225 completely absorbed into the trunks: A+I+O, EB, I+Ca and EB+A. Among these, I+Ca exhibited
226 the fastest injection speed (it was completely absorbed within 14 days) followed by A+I+O and
227 EB+A (23 days). However, only 77.5% of EB+CL and 56.7% of I were absorbed within 30 days.
228 In addition, the absorption rate of EB+CL showed no significant differences in all the measured
229 time points ($P > 0.05$). At the 9-day point, the quantities of A+I+O and I+Ca absorbed were the
230 largest (over 80% of the total).

231

232 ### Larval mortality

233 Larval mortality after 77 days of treatment differed significantly between insecticides ($F_{6,28} =$
234 23.721, $P < 0.001$), but neither the leaf position nor the interaction between these two factors was
235 significant ($F_{1,28} = 8.34$, $P = 0.007$; $F_{6,28} = 1.929$, $P = 0.111$). Larval mortality from the EB+CL
236 treatment was 100%, whereas mortality values from the A+I+O (0~33.3%), I+Ca (0), I
237 (16.7~50%) and EB+A (33.3~66.7%) treatments were not significantly different from that of the
238 control ($P > 0.05$); in fact, A+I+O and I+Ca caused no mortality (Fig. 4). Larval mortality 429
239 days after treatment differed significantly between insecticides ($F_{6,28} = 14.878$, $P < 0.001$), but

neither leaf position nor the interaction between these two factors was significant ($F_{1,28} = 0.031$, $P = 0.861$; $F_{6,28} = 0.454$, $P = 0.836$). Again, the mortality from the EB+CL treatment was 100%, while the data for A+I+O (16.7~33.3%) and I+Ca were not significantly different from the controls ($P > 0.05$), especially for I+Ca (mortality = 0). These results indicate that although I+Ca had a good absorption rate after application, it had no insecticide efficacy on the larvae.

Leaf loss

After 77 days of treatment, the percentages of damaged leaves were significantly different between insecticides, leaf position and the interaction between these two factors ($F_{6,28} = 19.439$, $P < 0.001$; $F_{1,28} = 43.969$, $P < 0.001$; $F_{6,28} = 8.921$, $P < 0.001$). The percentage of upper leaves damaged, in total, was approximately 20% or less for EB and EB+CL and significantly less than that for the other treatments ($P < 0.05$; Fig. 5) However, in comparison with the upper leaves, the data for lower damaged leaves for all the agents were significantly different from that of the controls ($P < 0.05$). The percentage of lower damaged leaves, in total, was less than 12% for EB+CL, A+I+O and EB (Fig. 3). Notably, the greatest contrast in damaged leaves from the upper (78.7%) and lower branches (8.2%) occurred with the A+I+O treatment. After 429 days, the percentage of damaged leaves was significantly different between insecticides ($F_{6,28} = 12.498$, $P < 0.001$), but neither the leaf position nor the interaction between these two factors was significant ($F_{1,28} = 3.603$, $P = 0.068$; $F_{6,28} = 0.76$, $P = 0.607$). The percentages of upper leaves damaged, in total, for EB+CL, EB, A+I+O and I were not significantly different from that of the control ($P > 0.05$); however, they were below the percentages from the other insecticides. The percentages of lower damaged leaves for EB+CL, EB and A+I+O were less than those from I and from the controls ($P < 0.05$).

Larval frass

After 77 days of treatment, the frass amount differed significantly between insecticides ($F_{6,28} = 44.768$, $P < 0.001$), but neither the leaf position nor the interaction between these two factors was significant ($F_{1,28} = 1.837$, $P = 0.186$; $F_{6,28} = 0.424$, $P = 0.857$). For all the treatments (except I+Ca), the frass amounts were smaller than controls ($P < 0.05$; Fig. 6). For EB+CL and EB, the data were more obvious. After 429 days of treatment, the frass amount was significantly different between insecticides and leaf position ($F_{6,28} = 65.478$, $P < 0.001$; $F_{1,28} = 15.061$, $P < 0.001$), but the interaction between these two factors was not significant ($F_{6,28} = 4.935$, $P = 0.0015$). The

frass amounts for EB and EB+CL were smaller than those found in the other treatments ($P < 0.05$). The frass amount from larvae on the upper leaves with I+Ca was significantly different from that of the controls ($P < 0.05$); however, for larvae on lower leaves, the frass amount was not significantly different from the controls ($P > 0.05$).

Finally, the absorption rates of the insecticides, leaf loss, and the mortality and frass amounts of *L. lepida* larvae were evaluated. The results are shown in Table 2. The EB treatment performed the best, followed by the EB+CL treatment. However, the injection of the latter was slightly less effective.

DISCUSSION

The selection of appropriate trunk injection agents is key for the successful implementation of trunk injection technology (*Dedek et al., 1986*; *Takai et al., 2004*). For a no-pressure injection system (where the only pressure in the system is that of gravity), it is important for the liquid chemicals in external injection plastic bottles to move into the plants quickly; in other words, this is the first indication of how well the liquid chemicals have been absorbed after application. No-pressure injection systems such as the method used here may seem to be less advantageous because their lack of pressure can make the uptake slow; however, they are inexpensive and simple to use. Here, we found that four insecticides (i.e., A+I+O, EB, I+Ca and EB+A) were completely absorbed into the trunks within 30 days; additionally, more than 80% of A+I+O and I+Ca were absorbed into the trunks after only 9 days. However, for 4% imidacloprid, only 56.7% of the agent was absorbed within 30 days, and its insecticide efficacy on the mortality of *L. lepida* larvae was poor. In contrast, the conductivity and insecticide efficacy of imidacloprid on avocado groves (*Byrne et al., 2012*) and ash trees (*Mota-Sanchez et al., 2009*) were acceptable, although the authors did not mention whether the chemicals were completely absorbed into the plants after application. The reason for this result may be that chemical conductivity was affected by the injection time, procedure, tree size, growth, or even the type, concentration and formulation of the chemicals (*Harrell, 2006*; *McCullough et al., 2005*; *Cowles et al., 2006*; *Tanis et al., 2012*).

It is worth to note that larval mortality could be produced by using our trunk injection and bioassay method, because the insecticide residues had been detected in leaf samples collected from treated trees, e.g. 16.5~45.6 μg g^{-1} emamectin benzoate occurred in upper leaf tissues after half a year, although sample number was not enough for statistics and not showed in this study; while on the other hand, in the control group all the larvae were able to maintain normal

physiology state and carry out molt. Besides, we used the method of trunk injection to complete the conductance or movement of chemical compounds through the tree. The choice of application method was according to local climate conditions. In general, heavy rain occurs during April to early July in southeast of China. However, other methods such as leaf spraying and trunk painting are more vulnerable to heavy rain, which washes off the insecticide applied to leaves or trunks (*Ichinose et al., 2010*). Additionally, the mortality of target pests was significant fluctuation due to the different degree of rainfall following the application of leaf spraying (*Ichinose et al., 2010*). Meanwhile, the larval bioassay in our study was similar to leaf-residue method (*Busvine, 1980*), which contains host plant and is closer to the natural state of action mode.

We found that EB alone or mixed with other agents (i.e., EB+A and EB+CL) exhibited a better absorption rate and insecticide efficiency. Although only 77.5% of the total amount of EB+CL was absorbed into the injected trees, its insecticide efficiency, based on larval mortality, achieved a level as high as that of EB (>80%). We suggest that the mixture of EB and CL may have a synergistic effect. Interestingly, other chemicals such as A+I+O and I+Ca showed a better absorption rate but a lower insecticide efficiency. Specifically, larval mortality was zero in the I+Ca treatment group, and the surviving larvae could enter the molt stage. However, previous studies have reported that imidacloprid insecticides effectively control many groups of insects, such as sap-feeders and beetles, following trunk injection (*Jeschke & Nauen*, 2008; *Mota-Sanchez et al., 2009*). The reason for their failure here may be that 1) the chemical residue in the leaves was too low to be effective as an insecticide, or our trunk injection of A+I+O and I+Ca may not have provided sufficient volume for a duration of 77 days, and/or 2) although previous studies showed that chemical metabolites were toxic to target insects as well as the parent compound (*Nauen et al., 1998*; *Mota-Sanchez et al., 2009*), it is possible that the effective components of the chemical may be negatively impacted by plant metabolic processes.

Previous studies have shown that the concentrations of trunk-injected chemicals among plant tissue types were different among plants as a whole, but that leaves showed much greater concentrations (*Mota-Sanchez et al., 2009*; *Takai et al., 2004*; *Xu et al., 2004*). For example, the imidacloprid concentrations in leaves increased steadily throughout the first growing season and were highest in leaf tissues, also were detected in leaves in the year following the injection (*Mota-Sanchez et al., 2009*). Therefore, for leaf-feeding insect pests, leaf loss was negatively correlated with the chemical concentration in leaves. EB (emamectin benzoate) acts as an antagonist for gamma-aminobutyric acid-gated chloride channels, causing a disruption of nerve impulses and rapid paralysis in a range of Lepidopteran species (*Kass et al., 1980*; *Ishaaya et al., 2002*). In addition, it has excellent control effects on nematodes (*Kazuya et al., 1999*; *Cheng et*

al., 2015) and emerald ash borers (*Flower et al., 2015*) through either trunk or soil injections. Similarly, in our study, we found that the conductivities of both EB+CL and EB were acceptable, and they also had a longer duration of insecticide efficiency (429 days). However, another mixed agent, EB+A, showed insecticide efficiency only on the lower leaves and failed to persist over time. This result may have occurred because the different agent mixtures had different active ingredients in different concentrations. In the A+I+O treatment group, leaf loss from the lower canopy was less than that from the upper canopy, which indicates that higher concentrations of the agent were retained primarily in the lower leaves.

The amount of frass excreted by the insect pests can be used as the main indicator for estimating whether an insecticide is efficient (*Paguia et al., 1980*; *Yang et al., 2006*). In the present study, we found that the larval frass was affected to various degrees by all the treatments except for I+Ca; however, the frass amounts from the EB and EB+CL treatment groups were below those of the other treatments, which suggests that such chemical agents may have a stronger insecticidal effect on larvae. A previous study demonstrated that a decrease in food uptake was significantly correlated with decreased frass in insect pests (*Yang et al., 2006*). This result corroborates our previous investigation (not included in this study), in which we found that the amount of frass was significantly positively correlated with the extent of leaf damage. Interestingly, insects can reduce the toxicity of chemical agents through an excretion mechanism (*Bues et al., 2005*; *Liu et al., 2006*). Therefore, the detection and analysis of frass could be an important method for further estimating the metabolic residues of injected chemical agents. However, the efficacy of insecticides based on the mortality of the targeted *L. lepida* is the most important prerequisite for choosing suitable trunk-injection insecticides.

CONCLUSION

Overall, we conclude that emamectin benzoate (EB) and emamectin benzoate + clothianidin (EB+CL) trunk-injected insecticides were rapidly absorbed into *O. fragrans*, showed significant insecticide efficiency, and remained effective over a longer duration than the other insecticides. However, the safety of these injection insecticides on the flowers of *O. fragrans* must be further studied in future research.

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485

Table 1(on next page)

Active ingredients and their formulation for the trunk injection.

Active ingredients and their formulation for the trunk injection.

1 Table 1. Active ingredients and their formulation for the trunk injection.

Trunk injection chemicals (abbreviation or code)	Formulation or composition	Active ingredient percentage (%)
EB+CL	Emamectin benzoate + clothianidin	10
A+I+O	Abamectin + imidacloprid + omethoate	21
EB	Emamectin benzoate	2.5
I	Imidacloprid	4
I+Ca	Imidacloprid + carbosulfan	4
EB+A	Emamectin benzoate+abamectin	2.5

2

3

Figure 1(on next page)

Structure diagram of the plastic bottle.

Structure diagram of the plastic bottle (injection device). Two main parts of the bottle, i.e. bottle body (containing chemicals) and bottle tip (inserting the drilled hole).

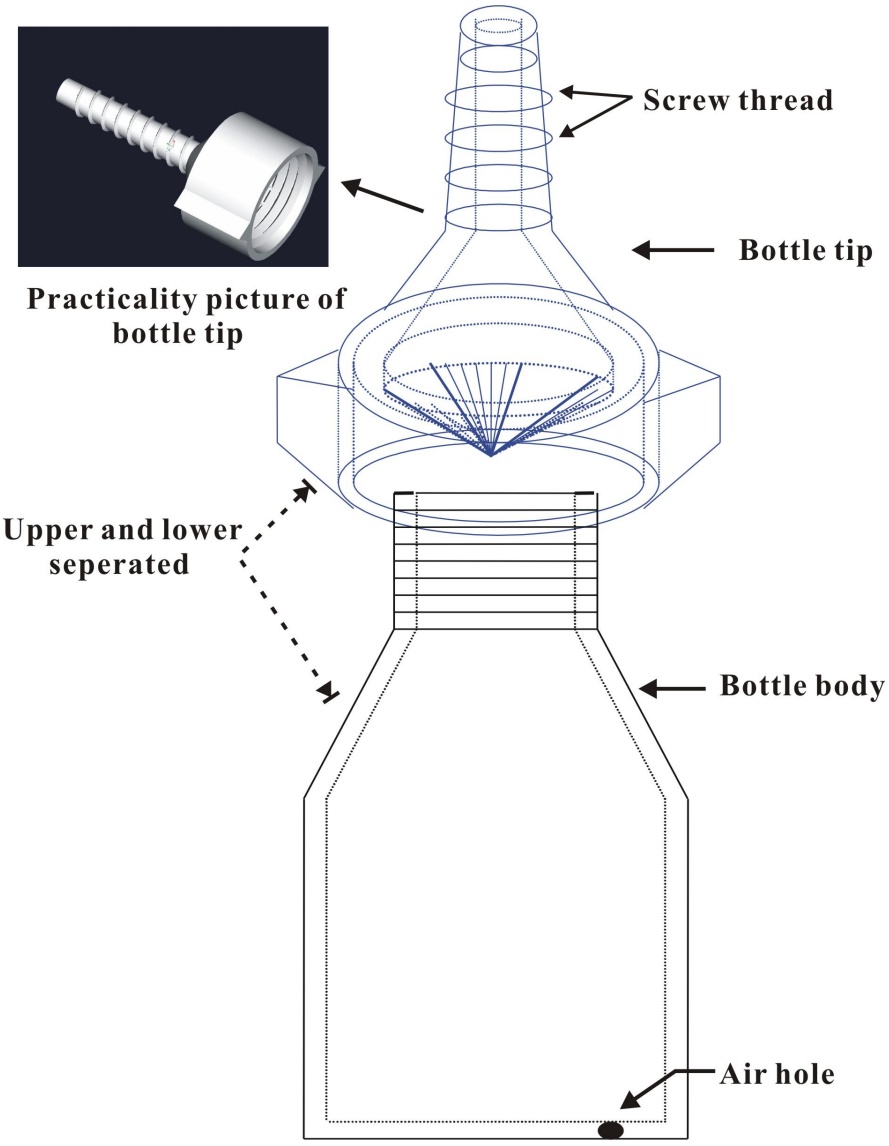

Practicality picture of bottle tip
Screw thread
Bottle tip
Upper and lower seperated
Bottle body
Air hole

Figure 2(on next page)

A diagram of the setup for larval bioassay.

A diagram of the setup for larval bioassay.

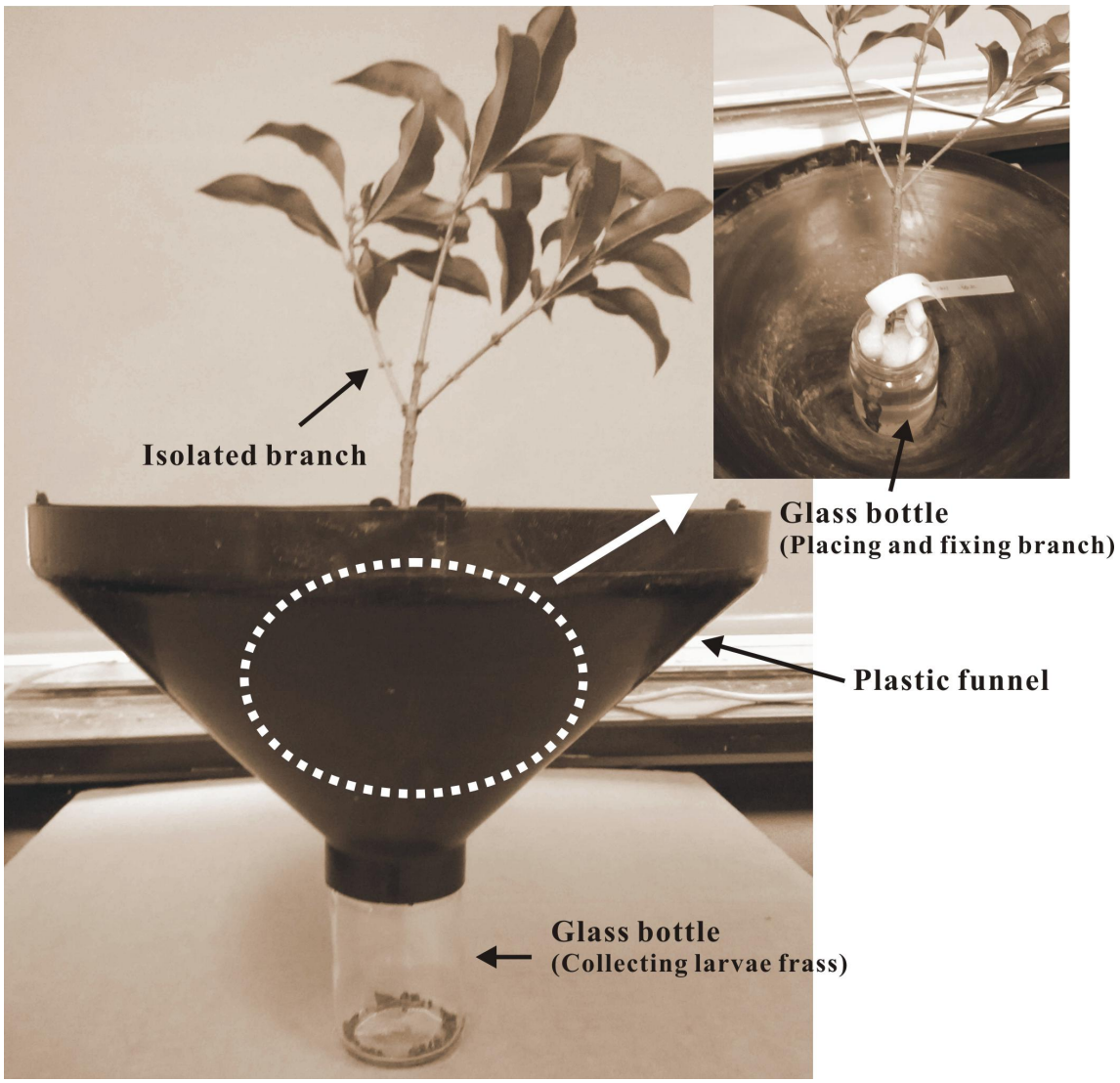

Isolated branch
Glass bottle
(Placing and fixing branch)
Plastic funnel
Glass bottle
(Collecting larvae frass)

Figure 3(on next page)

Absorption rate of insecticides

<!--[if !supportAnnotations]--> <!--[endif]--> Mean (±SE) percentage (%) of the six types of insecticides (EB+Cl, A+I+O, EB, I, I+Ca, and EB+A) absorbed into the trunk of *Osmamthus fragrans* at 9, 14, 23, and 30 days after application of trunk injection. $N = 18$ trees. Insecticides (between-subjects) and observation time (within-subjects) effects were significant ($P < 0.001$); Insecticides ´ observation time interaction effect was not significant. Bars labeled with different lowercase letters are significantly different at $P = 0.05$ from each other in the same insecticide group based on Dunn's range test.

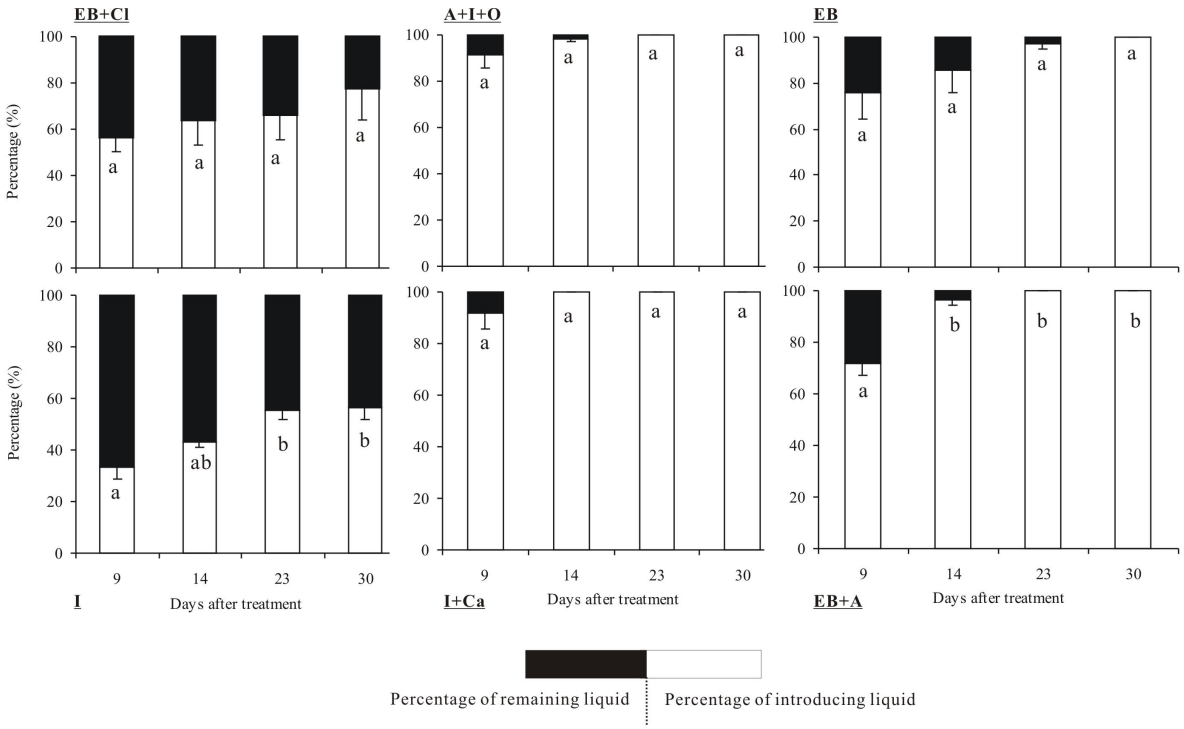

EB+Cl
A+I+O
EB
I
I+Ca
EB+A
Percentage (%)
Days after treatment
9
14
23
30
Percentage of remaining liquid
Percentage of introducing liquid

Figure 4(on next page)

Larval mortality

<!--[if !supportAnnotations]--> <!--[endif]--> Mean (±SE) mortality of *Latoia lepida* larvae on upper and lower leaves collected from *Osmamthus fragrans* trees treated with the insecticides of EB+CL, A+I+O , EB, I, I+Ca, and EB+A, by trunk injection after 77 and 429 days, respectively. $N = 42$ isolated branches in each observation time. Any observation time, insecticides effect was significant ($P < 0.001$); leaf position and insecticides × leaf position interaction effects were not significant. Bars labeled with different lowercase or uppercase letters are significantly different at $P = 0.05$ from each other in the same leaf layer group (upper or lower leaves) based on Dunn's range test.

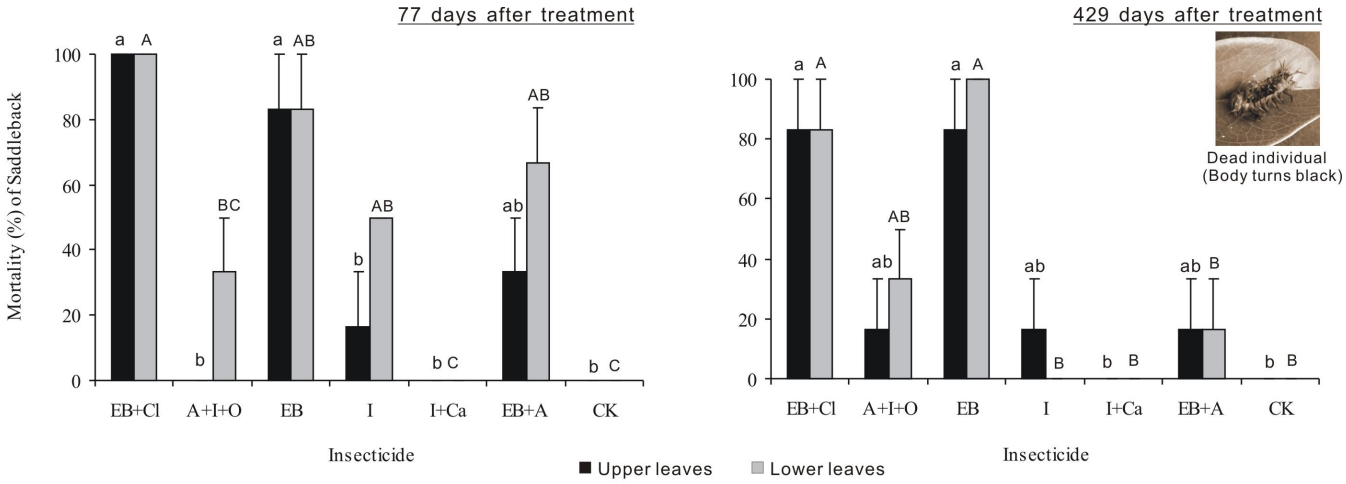

77 days after treatment
429 days after treatment
Mortality (%) of Saddleback
100
80
60
40
20
0
a A
b
BC
a AB
b
AB
b C
ab
AB
b C
a A
ab
AB
a A
ab
B
b B
ab B
b B
EB+Cl
A+I+O
EB
I
I+Ca
EB+A
CK
Insecticide
Upper leaves
Lower leaves
Dead individual
(Body turns black)

Figure 5(on next page)

Percentage of damaged leaves

Mean (±SE) percentage of damaged leaves collected from upper and lower canopies after the application of six types of trunk injection at 77 and 429 days. $N = 42$ isolated branches in each observation time. 77 days after treatment, insecticides, leaf position, and insecticides ´ leaf position interaction effects were significant ($P < 0.001$); 429 days after treatment, insecticides effect was significant ($P < 0.001$), and leaf position and insecticides × leaf position interaction effects were not significant. Bars labeled with different lowercase or uppercase letters are significantly different at $P = 0.05$ from each other in the same leaf layer group (upper or lower leaves) based on Dunn's range test.

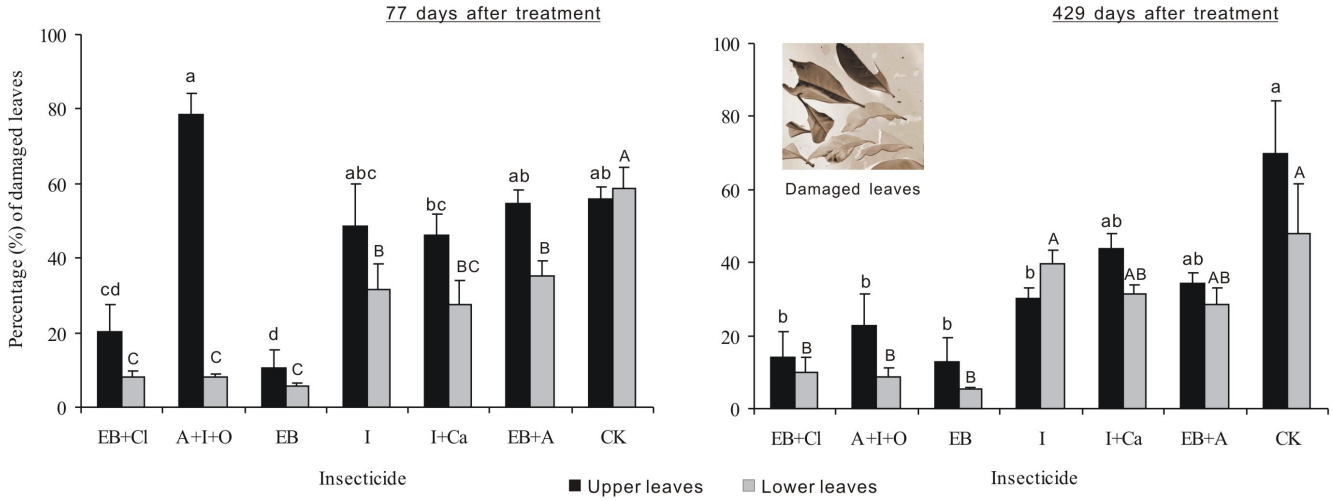
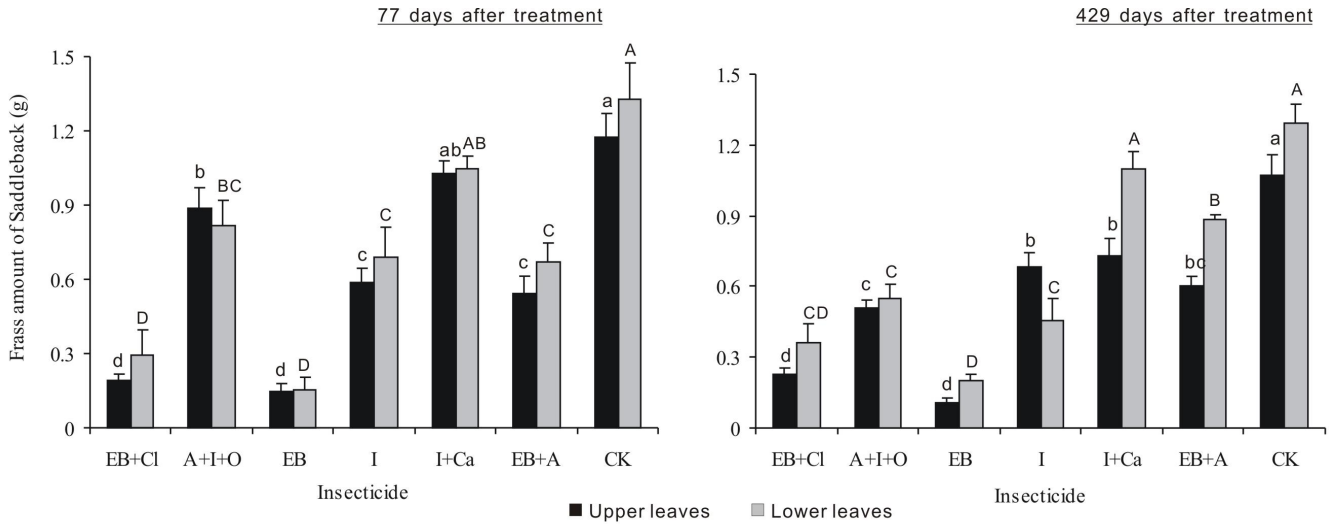

77 days after treatment
429 days after treatment
Percentage (%) of damaged leaves
Damaged leaves
EB+Cl
A+I+O
EB
I
I+Ca
EB+A
CK
Insecticide
Upper leaves
Lower leaves

Figure 6(on next page)

Frass amounts of *L. Lepida* larvae

Mean (±SE) frass amounts of *L. Lepida* larvae fed on upper and lower isolated leaves for 5 consecutive days after the application of six types of trunk injection at 77 and 429 days. $N = 42$ isolated branches in each observation time. 77 days after treatment, insecticides effect was significant ($P < 0.001$), and leaf position and insecticides × leaf position interaction effects were not significant. 429 days after treatment, insecticides and leaf position effects were significant ($P < 0.001$), and insecticides × leaf position interaction effect was not significant. Bars labeled with different lowercase or uppercase letters are significantly different at $P = 0.05$ from each other in the same leaf layer group (upper or lower leaves) based on Dunn's range test.


77 days after treatment
429 days after treatment
Frass amount of Saddleback (g)
1.5
1.2
0.9
0.6
0.3
0
d
D
b
BC
d
D
c
C
ab
AB
c
C
a
A
d
CD
c
C
d
D
b
C
b
A
bc
B
a
A
EB+Cl
A+I+O
EB
I
I+Ca
EB+A
CK
Insecticide
Upper leaves
Lower leaves