Aphids, or plant lice, are small, sap-sucking insects (Paik 1972). They are among the most important insect pests of many crops worldwide and cause damage that lowers plant quality and results in reduced crop production (Kim et al. 2011, Baek et al. 2013). *Pseudoregma bambucicola* (Takahashi, 1921) is a parthenogenic aphid species (holocyclic) that is widely distributed throughout the warmer regions of eastern Asia. This species infests *Bambusa* bamboo stems, branches, twigs, leaves, and shoots, developing into large and high-density colonies that impede bamboo growth (Fukatsu et al. 2001, Ijichi et al. 2004). The delicate branches of infested bamboo turn brown and die, while the aphid infestation also induces sooty mold growth, the mold of which inhibits photosynthesis. *P. bambucicola* has a very high reproductive potential and can cause substantial injury and even death to young plants (Petitt and Smilowitz 1982).

The prevention and control of aphids involves two commercially available management tools: insecticidal seed treatments and treatments that induce host plant resistance. The control of *P. bambucicola*, in particular, involves the application of chemicals, such as imidacloprid, to which many species are resistant. In this study, we isolate a novel botanical pesticide (9-oxo-10,11-dehydro-ageraphorone) from an *Eupatorium adenophorum* (Asteraceae: Compositae) petroleum ether extract and test the aphicidal activity of this compound against *P. bambucicola* in laboratory bioassay and field-based experiments. This ageraphorone compound at a concentration of 2 mg/ml caused 73.33% mortality (corrected mortality: 72%) of *P. bambucicola* by laboratory bioassay within 6 h. Even at lower concentrations, this compound caused greater 33% mortality (corrected mortality: 30%) of aphids. Field experiments with naturally infested bamboo plants showed that two applications of 2 mg/ml ageraphorone to infested plants completely cleared infestations within 30 d. These effects were similar to those of the positive control (imidacloprid). These results reveal that 9-oxo-10,11-dehydro-ageraphorone exhibits significant aphical activity against bamboo aphids. We suggest that future research will be directed at developing this ageraphorone compound from *E. adenophorum* as an aphicidal agent for biocontrol.

Materials and Methods

Ethics Statement. No specific permissions were required for the activities conducted in this study. The location is neither privately owned nor protected. The experiments did not involve endangered or protected species.

Plants and Aphids. *E. adenophorum* was collected in Xichang City, Sichuan province, China. The aerial parts of *E. adenophorum* were air-dried and then crushed in a knife mill. Morphological identification of the plant was based on a taxonomic key (Li 1998). Aphids were collected from a bamboo tree naturally infested with *P. bambucicola*. The laboratory bioassay experiments used wingless parthenogenetic *P. bambucicola* obtained from the bamboo plant. The field experiments involved the direct treatment of insects on the naturally infested bamboo plant.

Isolation and Identification of Ageraphorone. The aerial parts of *E. adenophorum* were dried and crushed in a knife mill. Subsequently, the resulting residue was extracted with petroleum ether and the petroleum ether extract was concentrated. The concentrate was partitioned into petroleum ether and ethyl acetate. The ethyl acetate fraction was further purified using silica gel column chromatography. The ageraphorone compound was isolated at a concentration of 9-oxo-10,11-dehydro-ageraphorone. The structure of the ageraphorone compound was identified by nuclear magnetic resonance and mass spectrometry.

Key Words: Aphicidal, *Pseudoregma bambucicola*, bamboo aphid, biocontrol, botanical, insecticidal
8 kg of *E. adenophorum* plant material was soaked in 95% ethanol (600 ml of 95% ethanol per 100 g plant material) for 30 min at room temperature. The material was then extracted twice with boiling ethanol under reflux, this step was 1 h per cycle. Then, the extracts were combined and concentrated by evaporation using a rotary evaporation apparatus. Finally, the ethanol extract was obtained. Then, we extracted ethanol extract with petroleum ether and obtained the petroleum ether extract of *E. adenophorum* (Nong et al. 2014a). The petroleum ether extract was then chromatographed on a silica gel column using a gradient of ratios of petroleum ether to acetone (50:1 — 5:1) as the eluents to obtain the compound. We used 1H NMR and 13C NMR (Wang et al. 2007) to identify the structures of the compound obtained from the *E. adenophorum* petroleum ether extract. The compound was subsequently identified as 9-oxo-10,11-dehydro-ageraphorone by nuclear magnetic resonance (NMR). NMR spectra were measured with a Bruker DRX-400 instrument with tetramethylsilane as the internal standard (Nong et al. 2014b).

**Aphicidal Activity of Ageraphorone to *P. bambucicola* With Laboratory Bioassay.** This was performed according to the methods of Nong et al. (2012, 2014a) and Chemenskaya et al. (2012) with some modifications. Glycerin and distilled water (1:1) were used for solvent preparation. The ageraphorone compound was used at four different concentrations (2.0, 1.0, 0.5, and 0.25 mg/ml). We added 2 ml of each concentration to a Petri dish (10 cm in diameter) containing filter paper. The filter paper was then placed on the filter paper in each Petri dish and incubated at 20 ± 2°C and 80 ± 10% relative humidity (Chemenskaya et al. 2012). Three replicates were performed for each concentration. The viability of the *P. bambucicola* insects was checked regularly by needle stimulation and aphids that displayed no reaction were recorded as dead. Imidacloprid was used as the positive control, whereas glycerin and distilled water (1:1) were used as negative controls.

**Aphicidal Activity of Ageraphorone to *P. bambucicola* in vivo (Field Experiment).** The field experiment was performed in a bamboo grove where severe infestation by *P. bambucicola* was observed. Each treatment was repeated thrice. We selected nine bamboo trees showing *P. bambucicola* infestation, these nine infested plants were chosen that appeared to have comparable infestations were chosen and then these were randomly assigned to treatments. These nine bamboo plants were randomly divided into three groups (A, B, and C). Plants in group A were treated with 2 mg/ml of 9-oxo-10,11-dehydro-ageraphorone (treatment group), whereas plants in group B were treated with imidacloprid (positive control group), and plants in group C were administered with glycerin and distilled water (1:1) (negative control group). All nine bamboo plants were treated twice on days 0 and 4. We used a small sprayer (especially used for thin-layer chromatography) for the application of ageraphorone, imidacloprid, and glycerin. Spraying approximately 20 ml of each substance on each bamboo tree appeared to distribute the chemicals on the plant and cover the aphids. Observations were conducted on day 0 (prior to spraying) and on days 4, 8, 12, and 30, using either a three-point or one-point sampling strategy. On days 0 and 4, we sampled three points on the plant (the upper, middle, and lower parts of each aphid gathering area). On days 8, 12, and 30, we only sampled one of those three areas. Sampling aphids involved collecting the insects from a 1 cm² area per point on the plant to calculate the mean aphid number. The aphid reduction rate and field treatment effect were calculated using the following formulas (Xu et al. 2009):

1. Aphid reduction rate (ARR, %) = (number of aphid before spraying – number of aphid after spraying)/number aphid before spraying × 100%.
2. Field treatment effect (%) = (ARR of treated group – ARR of control group)/(100 – ARR of control group) × 100%.

**Statistical Analyses.** Analysis of variance tests were conducted using SAS software (SAS Institute 2002) to assess the significance of differences in insect mortality rates under the different ageraphorone concentrations. The median lethal time (LT50) was calculated using a complementary log-log model. We also assessed the statistical significance of field experiment results, mainly the field treatment effect (%) of different concentrations of extracts and different treatment times were considered statistically significant when *P* < 0.05. The significance values were corrected with Duncan’s multiple comparisons test.

**Results**

**Identification of the Aphicidal Compound.** The structure of the active compound was identified by comparing its NMR data with data from literature. The NMR spectra of the compound showed the presence of one C = CH group (δH 6.23, 1H, br s; δC 135.8 s, 146.3 d), four methyl groups (δH 2.03, 1.87, 1.71, each 3H, s; δH 0.96 3H, d, J = 6.8 Hz), and two carbonyl groups (δC 197.7 s, 202.8 s) (Nong et al. 2014b). The spectral data were in agreement with published data (Shi et al. 2012) and this compound was identified as 9-oxo-10,11-dehydro-ageraphorone (molecular formula C15H20O2; Fig. 1).

**Aphicidal Activity of Ageraphorone Against *P. bambucicola* With Laboratory Bioassay.** We found that 9-oxo-10,11-dehydro-ageraphorone was highly toxic to *P. bambucicola* (Table 1). The 2 mg/ml concentration of the compound caused 73.33% (corrected mortality [subtracted the mortality of the negative control]; 70%) mortality among aphids within 6 h. Even concentrations of 1.0 and 0.5 mg/ml of the ageraphorone compound caused 60% and 33.33% (corrected mortality; 57% and 30%) aphid mortality, respectively (Table 1).

**Toxicity Analysis of Ageraphorone With Laboratory Bioassay (Median Lethal Time, LT50).** The toxicity of ageraphorone extract against *P. bambucicola* was tested with laboratory bioassay using a complementary log-log model. The data demonstrate that the extract of ageraphorone from *E. adenophorum* has a strong toxic effect against *P. bambucicola*. The probit regression analysis by regression line of different concentration of ageraphorone show that the toxicity of ageraphorone has to be time- and concentration dependent. The LT50 values of the 2.0, 1.0, and 0.5 mg/ml concentrations of ageraphorone were 4.5, 5.4, and 8.9 h, respectively. The laboratory bioassay aphicidal activity of 9-oxo-10,11-dehydro-ageraphorone showed dose and time dependence (Table 2).

**Field Experiment.** Prior to the field experiment, infested plants that appeared to have comparable infestations were chosen and then these were randomly assigned to treatments. Aphid density area was greatly reduced in group A (ageraphorone group) on days 4 and 8 postspaying (Fig. 2A2 and A3), and by day 12, nearly all of the infested plants in this group had completely recovered. Moreover, no recurrence of infestation was observed on day 30 in the plants in this group.

Infested bamboos in the positive control group (group B, treated with imidacloprid) also exhibited improvement during the experimental period with enhanced recovery after the second treatment (Fig. 2B2 and B3). As expected, bamboo plants in the negative control group (group C, treated with glycerol and water) showed no signs of recovery (i.e.,...
Table 1. The aphicidal activity of 9-oxo-10,11-dehydro-ageraphorone against *P. bambucicola* with laboratory bioassay

| Different concentration of ageraphorone | Mean mortality (%) ± SE of each observation time (min) |
|----------------------------------------|------------------------------------------------------|
|                                        | 120 min                               | 180 min                               | 300 min                               | 360 min                               |
| 2 mg/ml (ageraphorone)                 | 16.67 ± 3.33b(b)                      | 23.33 ± 3.33b(b)                      | 50.00 ± 10.00a(b)                     | 73.33 ± 6.67ab(b)                     |
| 1 mg/ml (ageraphorone)                 | 13.33 ± 3.33bc(b)                     | 26.67 ± 3.33bc(b)                     | 40.00 ± 5.77b(ab)                     | 60.00 ± 5.77ab(b)                     |
| 0.5 mg/ml (ageraphorone)               | 3.33 ± 3.33cd(b)                      | 13.33 ± 6.67bc(b)                     | 23.33 ± 3.33ab(b)                     | 33.33 ± 6.67ab(b)                     |
| 0.25 mg/ml (ageraphorone)              | 3.33 ± 3.33cd(b)                      | 13.33 ± 6.67bc(b)                     | 23.33 ± 3.33ab(b)                     | 33.33 ± 6.67ab(b)                     |
| Positive (imidacloprid)                | 40.00 ± 5.77b(a)                      | 46.67 ± 3.33b(a)                      | 56.67 ± 6.33b(a)                      | 83.33 ± 3.33b(a)                      |
| Untreated (glycerin and distilled water 1:1) | 0.00 ± 0.00d(d)                  | 3.33 ± 3.33d(c)                      | 3.33 ± 3.33d(c)                      | 3.33 ± 3.33d(c)                      |

Ten *P. bambucicola* aphids and three replicates were performed for each concentration. SE: standard error of means. Different lower case letters within a row denote significant differences between different times (*P* < 0.05). Different lower case letters in brackets within a column denote significant differences between different concentrations (*P* < 0.05).

Table 2. The probit regression analysis of toxicity (LT50) of ageraphorone against aphids with laboratory bioassay

| Different concentration of ageraphorone | Regression line | LT50/(h) (95% FL) |
|----------------------------------------|-----------------|-------------------|
| 2 mg/ml                                | \( Y = 3.256X - 2.117 \) | 4.5 (3.8–5.6)     |
| 1 mg/ml                                | \( Y = 2.609X - 1.907 \) | 5.4 (4.3–8.4)     |
| 0.5 mg/ml                              | \( Y = 2.614X - 2.486 \) | 8.9 (6.3–34.9)    |

Regression line: the equation reflects the relationship between the toxicity and the concentration of ageraphorone; LT50, median lethal time value; 95% FL, the overall parameter is 95% in this range.

Fig. 2. Observations of the field treatment effects on infected bamboos. Plants in group A were treated with 2 mg/ml of the ageraphorone extract from *E. adenophorum*. Plants in group B were treated with imidacloprid, whereas plants in group C were untreated (treated with glycerine and distilled water 1:1). The photos A1, B1, and C1 show aphid infestations prior to “treatment.” The photos A2, B2, and C2 show observations of typical infestations on day 4 posttreatment, whereas photos A3, B3, and C3 show observations of typical infestations on day 8 posttreatment.
The field experiment was performed in a bamboo grove where severe infestation by *P. bambucicola*. Nine bamboo plants were randomly divided into three groups (A, B, and C). Plants in group A were treated with 2 mg/ml of 9-oxo-10,11-dehydro-ageraphorone, whereas group B were treated with imidacloprid, and plants in group C were administered with glycerin and distilled water (1:1). SE, standard error. Field treatment effect (%) = (aphid reduction rate of treated group – aphid reduction rate of control group)/(100 – aphid reduction rate of control group).

### Discussion

To discover new natural products requiring screening candidate plants, obtaining new active ingredients, and isolating and identifying the active plant constituents (Chermenskaya et al. 2012). We applied this procedure to obtain the compound 9-oxo-10,11-dehydro-ageraphorone from *E. adenophorum* petroleum ether extract. Previous studies have shown that oral administration of 9-oxo-10,11-dehydro-ageraphorone can cause hepatotoxicity in mice and antifeedant activity in *Helicoverpa armigera* (Bhurday et al. 2001, Shi et al. 2012). However, few studies have investigated the effect of compound 9-oxo-10,11-dehydro-ageraphorone against aphids. In this study, we used the methods of laboratory bioassay and field experiments, and the results showed that this compound exhibits significant aphidicidal activity against bamboo aphids.

Studies have shown that the main active ingredients of *E. adenophorum* include alkaloids, monoterpenoids, flavonoids, sesquiterpenes, and phenols, and these compounds are mainly concentrated in the leaves (He et al. 2006, Yan et al. 2006). Of these compounds, alkaloids and phenols, and these compounds are mainly concentrated in the plant. However, further studies should first verify the toxicity of ageraphorone for medicinal purposes. Integrated insect control methods using plant extracts combined with conventional pesticides present a sustainable means of controlling pest species (Xu et al. 2009). *E. adenophorum* extracts offer a natural pesticide that can be used as an alternative to chemical pesticides. As this weedy species is readily available, it can potentially reduce the costs of pest control. With further research, increasing numbers of botanical pesticides will be identified for use in biological control.

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