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RESEARCH ARTICLE

Antifeedant activity of gutta-percha against larvae of the *Hyphantria cunea* and *Anoplophora glabripennis*

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Eucommia ulmoides is a small tree that has evolved strong pest resistance. This study investigated the mechanism of this resistance by evaluating pest damage and antifeedant activity of the secondary metabolite gutta-percha. In the field, *E. ulmoides* displayed an average plant resistance of 93.3%, with most damage confined to leaves. We assessed the effects of gutta-percha as a feeding deterrent against *Hyphantria cunea* and *Anoplophora glabripennis* larvae by mixing it into or daubing it onto the surface of artificial diets. Diets containing more than 6% gutta-percha significantly reduced the amount of feeding by *A. glabripennis* larvae and the body length of *H. cunea* larvae. In addition, approximately 95% of the artificial diet was not consumed compared to the control without gutta-percha. The content, distribution, properties, and dynamics of gutta-percha in *E. ulmoides* indicate that gutta-percha may function as a physical-chemical barrier against insect pests.

Keywords: *Eucommia ulmoides*; investigation; antifeedant activity

Introduction

Secondary metabolites have a key role in plant resistance to pests and herbivores. Plant secondary metabolites can act as feeding deterrents or inhibit pest growth and development (Pavela & Herda 2007; Pavela et al. 2009; Rani & Murty 2009). Plants may adopt various chemical defenses, among them, the amount of defense chemicals intrinsic to the plant and not induced by the feeding of insects (Shi 1994; Maleck & Dietrich 1999).

Eucommia ulmoides is the only member of the family Eucommiaceae. It is a typical relict plant native to China, where now 95% of the *E. ulmoides* resources are located. It grows in most areas of China, and has a high economic value (Zhang & Xue 2011). Gutta-percha is a unique secondary metabolite of *E. ulmoides*; it is an isomer of natural rubber that has great industrial value. Gutta-percha is found in all *E. ulmoides* dry tissues, and accumulates to 3% in the leaves and 6% in the bark (Song et al. 2006). Following long-term investigations, it was discovered that *E. ulmoides* has a strong resistance to pests, and has rarely suffered disastrous pest infestations (Li 2002; Zeng 2004; Jiao & Fu 2009; Jiang 2012).

We investigated the mechanism of *E. ulmoides* resistance to *Hyphantria cunea* (Lepidoptera, Arctiidae), which is an invasive defoliator, and *Anoplophora glabripennis* (Coleoptera, Cerambycidae), which is a typical native borer that has co-evolved with domestic plants like *E. ulmoides*. Pest infestation patterns on *E. ulmoides* were investigated in Beijing, and the feeding-deterrent activity of Gutta-percha against the two species was studied. This will provide a base-line reference for future studies on the mechanism of pest resistance of *E. ulmoides*, and additional strategies for general pest control and prevention.

Materials and methods

Analysis of pest feeding

The investigation study was conducted in June 2014 in urban areas of Beijing. Five sample plots containing 30 *E. ulmoides* trees per plot were selected. Three distinct classes rating tree health were established to analyze pest infestation (Table A1) (Wu et al. 2002; Liu et al. 2003; Qie et al. 2013). Tree height, diameter, health status, and injuries were measured and recorded. The pest resistance percentage was calculated as follows: the number of pest-free trees/the total number of trees in the plot (Chen et al. 2008; Zhang et al. 2014).

Methods for determining the antifeedant activity of gutta-percha

Insect pests

H. cunea egg masses were provided by the China Academy of Forestry Sciences, Beijing. The second- and third-instars that hatched from the same egg mass were used. *A. glabripennis* larvae were collected from the Ningxia Province, China, from June to September, 2013, and 210 third-stage larvae that fed well on artificial diet were used. Both pests were fed artificial diets in an incubator with 25°C constant temperature, 75% humidity, and were starved for 3 days before experiments to ensure uniform feeding status.

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Chemical reagent

Gutta-percha (purity > 99%) was purchased from Sigma-Aldrich (182168-50G, USA), dissolved in petroleum ether (boiling in the range 30–60°C), and stored at 4°C.

Artificial diets

The artificial diet for *H. cunea* was provided by the Chinese Academy of Forestry, Beijing (Zhang et al. 2007). The diet for *A. glabripennis* was prepared in our lab according to previously described protocols (Zhao et al. 1999). At the end of diet preparation, it was poured into a 50 mL transparent plastic box and allowed to solidify. To feed *H. cunea*, the diet covered the bottom of the culture box. To feed *A. glabripennis*, the diet that had solidified in the 50 mL box was cut into four pieces (diameter = 6 cm, height = 2.5 cm). One piece was placed into each of four culture boxes as described in the next section.

Antifeedant activity of gutta-percha when mixed into diets

A. glabripennis larvae were separated into four groups with 10 larvae per group, and were fed in individual chambers with artificial diet containing 0%, 3%, 6%, or 9% gutta-percha for 5 days. Second-instars *H. cunea* were separated into four groups of approximately 50 larvae per group, and were fed in individual chambers with artificial diet containing 0%, 1%, 3%, or 6% gutta-percha for 10 days. The amount of feeding by *A. glabripennis* larvae was recorded, and the amount of feeding was graded to facilitate statistical analysis. No feeding was defined as class 1, while, 1–50% of the diet consuming was defined as class 2, more than 50% was defined as class 3. Ten *H. cunea* larvae were randomly selected to measure body length, and the average was calculated. All experiments were independently repeated three times.

In this initial study, we mixed petroleum ether in the artificial diets, and detected no effect on the feeding behavior of *H. cunea* or *A. glabripennis* larvae, which is probably due to the low-boiling point and fast evaporation of petroleum ether. Therefore, it was selected as the organic solvent for gutta-percha.

Antifeedant activity of a surface coating of gutta-percha

A. glabripennis larvae were separated into three groups of 10 larvae each, and every larvae was fed in individual chambers with artificial diet daubed with gutta-percha on the surface, or daubed on half of the diet and then covered with the remaining untreated diet (designated as interlayer), or not daubed (control), for 5 days. The amount of unconsumed diet was calculated as 'the number of larvae that failed to break through the gutta-percha layer/the total number of larvae'. Third-instar *H. cunea* larvae were separated into three groups of 30 larvae each, and were fed with artificial diet daubed with gutta-percha on the surface for 3 days. Then, the feeding area was measured using a 1 mm × 1 mm paper grid. The amount of unconsumed diet was calculated as 'the uneaten surface area of the diet / the total area of the diet' (Hu et al. 2011). All experiments were independently repeated three times.

Statistical analysis

Data were analyzed using SPSS v16.0 and Prism v6.0.

Results and discussion

Characteristics of pest damage to E. ulmoides

The height of E. ulmoides trees in the five sample plots was 6.3–11.5 m, and the diameter was 15.4–24.1 cm. Symptoms of pest feeding on the trees are summarized as described in Table A2. The percentage of trees that exhibited pest resistance averaged 93.3%, and only 1 *E. ulmoides* tree had poor health status (Class 3) out of the total 150 trees in all five plots. Most of the pest damage occurred on leaves. Only one *E. ulmoides* tree in Plot 4 had trunk damage by borers, and the area around the emergence hole was not covered by bark.

Antifeedant activity of gutta-percha when mixed into diets

The amount of feeding by *A. glabripennis* larvae significantly decreased in a dose-dependent manner when gutta-percha was mixed into the artificial diet (Figure A1). Some of the larvae in the 3% gutta-percha group apparently bored into the diet on the fifth day, and the feeding on that day increased. This behavior was not observed in the groups fed with diet containing 6% or 9% gutta-percha.

The length of *H. cunea* larvae fed with artificial diet containing gutta-percha dramatically decreased in a dose-dependent manner after day 4 of the experiment (Figure A2). The reduction of body length was most obvious in the group that was fed diet containing 6% gutta-percha. Compared with larval length in the control group (0% gutta-percha), there was no significant difference on day 10 in the groups fed 1% and 3% gutta-percha. The body length curves had fluctuations on days 4 and 8 due to molting. Analysis of variance indicated that the body length difference in the group fed with diet containing 6% gutta-percha compared with that of control was statistically significant ($P < .05$).

Antifeedant activity of a surface coating of gutta-percha

Both *H. cunea* and *A. glabripennis* larvae normally feed on the artificial diet. Daubing gutta-percha on the surface of the diet significantly deterred feeding for both insect species (Table A3). The antifeedant effect of gutta-percha was more effective if it was daubed on the surface than if it was daubed on half of the diet and then covered with the remaining untreated diet (designated as interlayer). The amount of unconsumed diet for both *H. cunea* and *A. glabripennis* larvae was more than 95%.

Conclusion

Gutta-percha is synthesized by a group of *E. ulmoides* cells that are linked into a web-like network. Gutta-percha has significant antifeedant activity against *H. cunea* and *A. glabripennis* larvae. The gutta-percha content in *E. ulmoides* consistently increases in parallel with the seasonal increase in pest activity (Cui et al. 1999). The gutta-percha content in different parts of *E. ulmoides* also varies. The content was higher in bark than in leaves (Tangpakdee 1997; Du et al. 2003), which may explain why more pest damage occurs in leaves than in bark. Higher gutta-percha contents in artificial diet had a stronger antifeedant activity against both larval species. Lower gutta-percha contents in artificial diet had limited antifeedant activity during the later stages of the experiment, which may be attributed to increased hunger and improved boring ability as the insects aged. Gutta-percha significantly deterred larval feeding when mixed into artificial diet at ≥6% concentration, or when daubed on the feeding surface of the diet. This indicates that gutta-percha deters insect feeding by acting as a physical or chemical barrier. Higher gutta-percha concentrations would generate stronger barriers.

E. ulmoides is an ancient plant, which shows stronger resistance to pests than trees that evolved after the Ice Age mass destruction. As a secondary metabolite, gutta-percha has no effect on growth and development of the plant, but plays an important role in pest resistance. The long-term co-evolution of *E. ulmoides* and pests provided *E. ulmoides* with this physical barrier (Turlings & Benrey 1998; Qin & Wang 2001; Zhang & Liu 2003; Zhao et al. 2006; Felton & Tumlinson 2008; Koul 2008), but how it evolved still requires further study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix

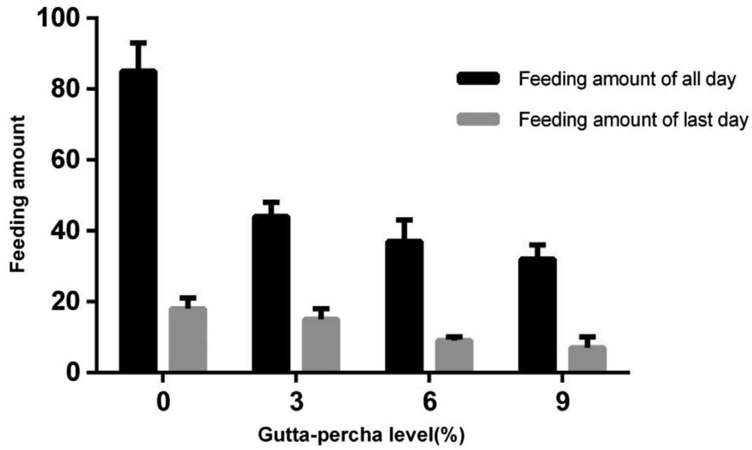

Figure A1. Analysis of artificial diet consumption by *A. glabripennis* larvae when diet contained 0–9% gutta-percha.

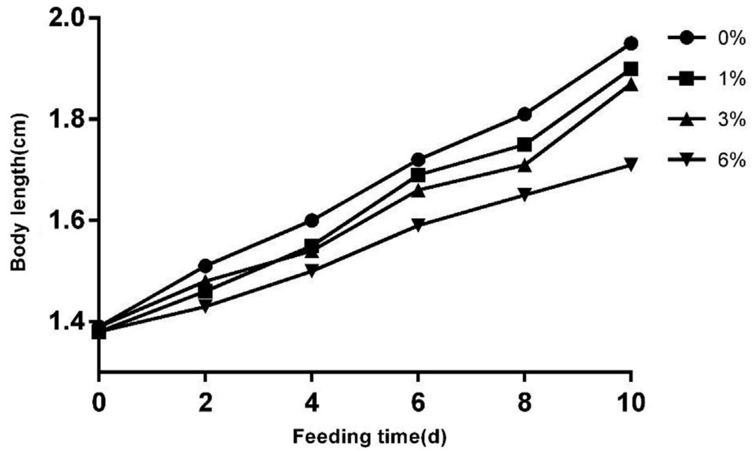

Figure A2. Analysis of *E. ulmoides* larval length after eating artificial diet containing 0–6% gutta-percha.

Table A1. Classification of pest feeding and damage on *E. ulmoides* trees.

Classification	Pest emergence or defecation holes	Leaves with symptoms of pest feeding (%)
Class 1	0	0
Class 2	1–5	1–50
Class 3	>5	>50

Table A2. The investigation on pests feeding traces of *E. ulmoides* in Beijing urban areas.

Tree species	Plot number	Average tree height (m)	Average tree diameter (cm)	Class 1*	Class 2*	Class 3*	Insect resistant (%)	Affected tissue
E. ulmoides	1	11.5	24.1	30	0	0	100.0	
	2	8.9	21.1	28	2	0	93.3	Leaf
	3	8.7	20.5	29	1	0	96.7	Leaf
	4	6.7	16.6	27	3	0	90.0	Leaf trunk
	5	6.3	15.4	26	3	1	86.7	Leaf

*Class 1, 2, and 3 are defined in Table A1.

Table A3. Analysis of artificial diet consumption by larvae after treating diet surfaces with gutta-percha.

Tested insects	Method of treatment with gutta-percha	Average per cent of unconsumed diet
Third-instar *Hyphantria cunea*	Surface	95.6^{a}
	Interlayer	81.3^{b}
	CK	0^{c}
Third-instar *A. glabripennis*	Surface	96.7^{a}
	Interlayer	73.3^{b}
	CK	13.3^{c}

Note: Different letters indicate statistically significant differences at the 5% level.