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INTERACTION BETWEEN *BISCHOFIA JAVANICA* BLUME (EUPHORBIALES: EUPHORBIACEAE) AND *COLOANA CINEREA* DWORAKOWSKA (HOMOPTERA: CICADELLIDAE): A PROTECTIVE ENZYME PERSPECTIVE

Zhen-De Yang[1,3], Yan-Juan Sun[1], Shu-Zhong Yu[1] and Xia-Lin Zheng[2,*]

[1]College of Forestry, Guangxi University, Nanning 530004, Guangxi Zhuang Autonomous Region, China
[2]College of Agriculture, Guangxi University, Nanning 530004, Guangxi Zhuang Autonomous Region, China
[3]Guangxi Colleges and Universities Key Laboratory of Forestry Science and Engineering, Nanning 530004, Guangxi Zhuang Autonomous Region, China
[*]Corresponding author email: zheng-xia-lin@163.com

ABSTRACT

Coloana cinerea Dworakowska (Homoptera: Cicadellidae) is a phloem-feeding pest on *Bischofia javanica* Blume (Euphorbiales: Euphorbiaceae), which is the main species of avenue tree in the urban landscape of China. Understanding their relationship is conducive to exploring the coevolution of both of them and the effective management of insect pests. In this study, their interaction was studied from a protective enzyme perspective. Results showed that Catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activities in *B. javanica* leaves were evidently enhanced by population density, feeding duration and their interaction of *C. cinerea*. CAT, POD, SOD, glutathione S-transferase (GST) and amylase (AMY) in *C. cinerea* were conversely affected by foliage maturity of *B. javanica*, feeding duration and their interaction. These changes in physiological compositions of both *C. cinerea* and *B. javanica* following stress or damage suggest that they have coevolved to an adaptation against each other.

Keywords: Landscape plant; Insect pest; Protective enzyme; Coevolution.

Abréviations: CAT, Catalase; POD, peroxidase; SOD, superoxide dismutase; GST, glutathione S-transferase; AMY, amylase.

INTRODUCTION

Plants and herbivores are constantly at battle in the progress of evolution. Plants have recognized damage via plant hormone signaling and evolved a diverse arsenal of direct and indirect defenses to reduce herbivore and the impacts of damage on plant performance, while herbivores evolve that they cope with changes in their host plants (Scott *et al*., 1998). On the one hand, reactive oxygen species (ROS) are constantly produced from plants, which has been considered to associate with plant recalcitrance against stresses, such as insect pest, drought, salinity, extreme temperatures and high irradiance (Cassells and Curry, 2001). Antioxidant enzyme system (including catalase, CAT; peroxidase, POD; superoxide dismutase, SOD) could resist the overproduction of ROS, which is detrimental in high concentrations (Cassells and Curry, 2001). On the other hand, protective enzyme system is also present in insects and plays an important role in the regulation of oxygen toxicity when they exposed to various abiotic and biotic stress factors (Wang *et al*., 2001). In general, the constitutive levels of the enzymes catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and amylase (AMY) correlated well with natural feeding habits of these insects and their relative susceptibility to prooxidant plant allelochemicals, flavonoid and xanthotoxin (a photoactive furanocoumarin).

Coloana cinerea Dworakowska, 1971 (Homoptera: Cicadellidae) is one of the most dangerous phloem-feeding pests of avenue tree in Guangxi, Guangdong and Taiwan provinces of China, Vietnam and India. In 2008, large area of *Bischofia javanica* Blume, 1825 (Euphorbiales: Euphorbiaceae), which is the main avenue tree in the urban landscape of China, was damaged by *C. cinerea* in Nanning and Zhongshan Cities, which brought about yellowing and falling of leaves, and reduced fructification and plant vigor (Chen *et al*., 2012; Li *et al*., 2012). Further investigation found that *C. cinerea* could damage about 20 species of landscape plants in Nanning City, and survival fed on *B. javanica* was higher (86.7%) than others (Sun, 2010). In the field, *C. cinerea* nymphs mainly aggregated on mature (functional leaf, 13.6 individual / leaf) more than tender leaves (incompletely expanded and laurel-green leaf, 3.5 individual / leaf) of *B. javanica* (Sun, 2010). Although the feeding style of *C. cinerea* causes comparatively little overt tissue damage, its infestations can cause a significant growth reduction. This is because plants are not only deprived of photosynthates (carbon) and free amino acids (nitrogen) (Tuomi *et al*., 1984) but also initiate metabolically costly defense responses (de Vos *et al*., 2007). Currently, studies concerning this species only

focused on the spatial distribution pattern (Chen *et al*., 2012) and biological characteristics (Li *et al*., 2012). However, there are no comprehensive studies investigating the interaction of both *C. cinerea* and *B. javanica*. Understanding their relationship is conducive to exploring the coevolution of both of them and the effective management of insect pests (e.g., breeding insect-resistant varieties and transgenic plants).

In this study, our objectives were to explore (1) the effect of *C. cinerea* on levels of protective enzyme in *B. javanica*, and (2) the effects of *B. javanica* on protective enzyme, GST and AMY activities in *C. cinerea*.

MATERIALS AND METHODS

Plant and insect materials: Seeds of *B. javanica* were procured from Guangxi Gaofeng Forestry Centre in 2008. Some seeds were sown in the experimental fields (Guangxi University, GXU), others were sown in plastic containers. These potting plants were arranged on the boundary of these experimental fields. All plants were fertilized with controlled release fertilizer, watered as required and not applied pesticides. These saplings in the fields and flowerpots were chosen as plant materials.

Nymphs of *C. cinerea* were collected on *B. javanica* in Nanning City (108°28´E, 22°84´N) from March to April, 2012. They were transferred to the potting plants in our laboratory. The colony was incubated at 28 ± 1 °C with a L15:D9 photoperiod, and a relative humidity of 60% - 80% to allow eclosion and oviposition. In this way, fifth instar nymphs from the laboratory population were obtained.

Assays of protective enzyme activities in *B. javanica* leaves after inoculation: In the fields, a 1-m-high net cage covering each *B. javanica* sampling were prepared, and 0 (control), 25 and 50 fifth instar nymphs were separately inoculated to the leaves on *B. javanica* saplings (30 cm height). Each treatment replicated three times. Fresh leaves were collected at 0, 3, 6 and 9 d after inoculation for determination the CAT, POD and SOD activities in leaves.

CAT activity was assayed with the titrimetric method described by Radhakissnan and Sarma (1963). POD activity was assayed spectrophotometrically with *o*-dianisidine as hydrogen donor (Sadasivam and Manickam, 1996). The assay of SOD activity was carried out with the method of Beauchamp and Fridowich (1971).

Assays of enzymes activities in *C. cinerea* after feeding on *B. javanica*: After starvation stress for 3 hours, fifth instar nymphs of *C. cinerea* reared separately with mature (functional leaf) and tender (incompletely expanded and laurel-green leaf) leaves of *B. javanica* collected from saplings in the field. After feeding for 0 (control), 2 and 4 h, nymphs were respectively frozen with liquid nitrogen as materials for the following experiment. Each treatment was replicated three times, and n = 60 for each treatment.

Nymphs from the six treatments ground to fine powder with a pre-chilled mortar and pestle with 1-mL physiological saline solution (0.9% NaCl). The extract was centrifuged for 20 min at 10,000 ×*g* and the supernatant was used for enzymatic assay. CAT, POD and SOD activities were determined according to the reagent kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu Province, China). Activities of GST and AMY were determined in accordance with previous procedures (Habig *et al*., 1974; Weidlich *et al*., 2013).

Statistical analysis: Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). The effects of population density and duration of feeding of *C. cinerea* and their interaction with the CAT, POD and SOD in *B. javanica* leaves, and effects of foliage maturity and duration of feeding and their interaction with the CAT, POD, SOD, GST and AMY in *C. cinerea* were analyzed using two-way ANOVA. A level of $P < 0.05$ was considered statistically significant.

RESULTS

Effects of *C. cinerea* on CAT, POD and SOD activities in *B. javanica*: Protective enzyme activities (i.e., CAT, POD and SOD) in *B. javanica* strengthened rapidly with the increasing of population density compared with control (Fig. 1A, B and C). The results of two-way ANOVA indicated a significant interaction between population density and feeding duration (Table 1).

Effects of *B. javanica* on enzymes activitiesin *C. cinerea*: The CAT in *C. cinerea* was significantly influenced by feeding duration, and interaction between foliage maturity and feeding duration (Table 2). Levels of POD and SOD were significantly influenced by foliage maturity (mature and tender leaves), feeding duration and their interaction (Table 2). Especially for those on the tender leaves, protective enzyme activities of nymphs sharply declined with the extending of feeding time ($P < 0.01$). Interestingly, enzymes activities of both CAT and POD in nymphs were gradually improved after feeding on mature leaves for two hours, and which indicated a significant difference ($P < 0.05$) (Fig. 1D and E).

Effects of foliage maturity and feeding duration on GST are similar to POD in *C. cinerea* nymphs (Fig. 2A). For example, the activity of GST was enhanced moderately after nymphs fed on mature leaves of *B. javanica* for 2 hours. Both foliage maturity and feeding duration affected the GST significantly according to the two-way ANOVA (Table 2).Although there is an obvious ascendant trend of AMY in nymphs feeding on mature

and tender leaves before 2 h, AMY activity suddenly decreased for those feeding on tender leaves after 2 h ($P < 0.01$) (Fig. 2B). Two-way ANOVA results illustrated that the interaction of both foliage maturity and feeding duration significantly influenced the AMY activity (Table 2).

Table 1. Effects of population density, duration of feeding and their interactions of *Coloana cinerea* on CAT, POD and SOD in *Bischofia javanica*. DF represents degrees of freedom. F represents F –value, and significance of results is showed by $P < 0.05$

Sources		CAT		POD		SOD		GST		AMY	
	DF	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
foliage maturity	1	4.119	0.065	212.757	<0.001	2050.325	<0.001	783.116	<0.001	13897.122	<0.001
duration of feeding	2	10.22	<0.05	154.418	<0.001	980.196	<0.001	1801.361	<0.001	8311.763	<0.001
foliage maturity× duration of feeding	2	7.475	<0.05	60.047	<0.001	614.496	<0.001	207.322	<0.001	4811.244	<0.001

Table 2. Effects of foliage maturity, duration of feeding and their interaction on CAT, POD, SOD, GST and AMY in *Coloana cinerea*. DF represents degrees of freedom. F represents F –value, and significance of results is showed by $P< 0.05$.

Sources		CAT		POD		SOD		GST		AMY	
	DF	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
foliage maturity	1	4.119	0.065	212.757	<0.001	2050.325	<0.001	783.116	<0.001	13897.122	<0.001
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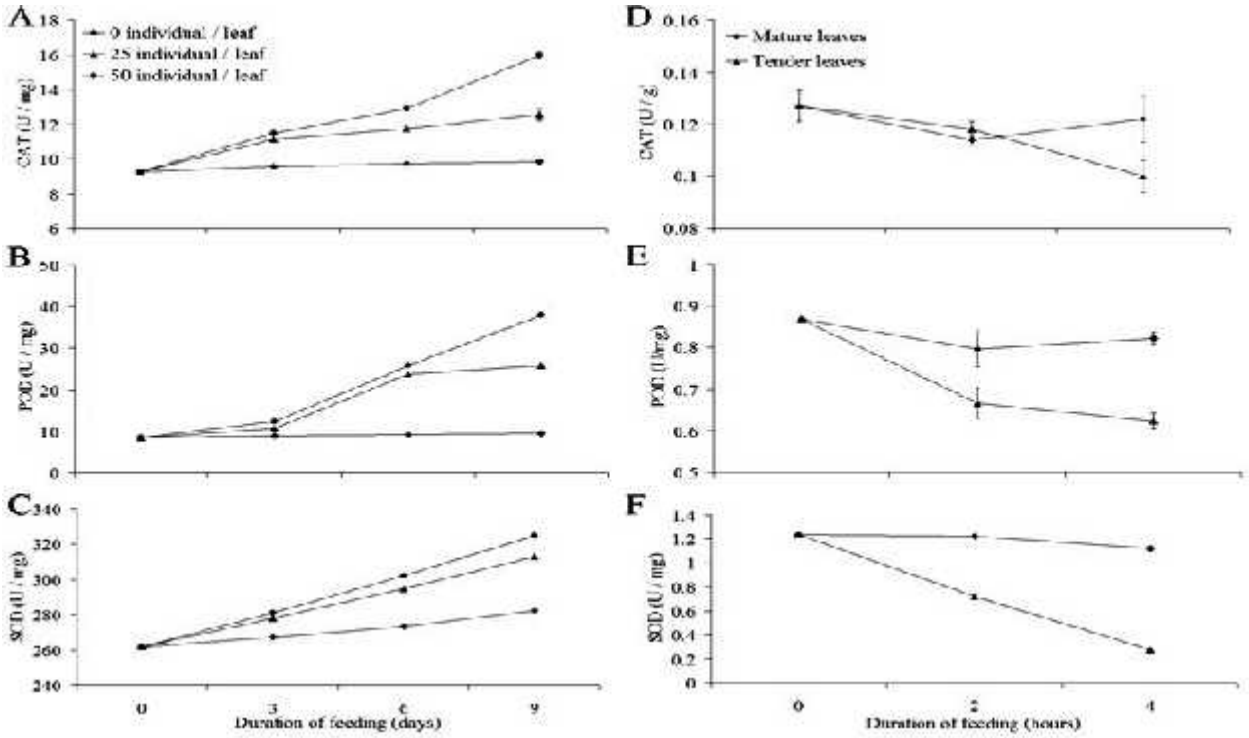

Figure 1. Effects of *Coloana cinerea* on CAT (A), POD (B) and SOD (C) in *Bischofia javanica*, and effects of *B. javanica* on CAT (D), POD (E) and SOD (F) in *C. cinerea*. Each treatment was three replications. Each replication was measured three times. Error bars indicate SE.

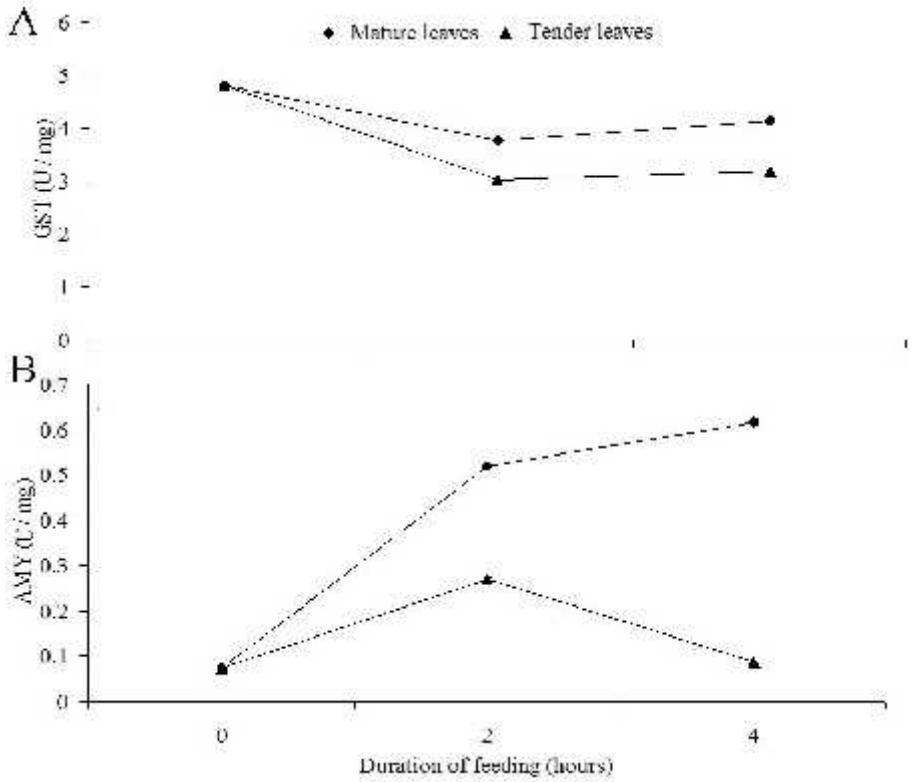

Figure 2.Effect of *Bischofia javanica* on GST (A) and AMY (B) in *Coloana cinerea*. Each treatment was three replications. Each replication was measured three times. Error bars indicate SE.

DISCUSSION

Induced responses (including active and passive responses) can increase the "resistance" of the plant to further herbivore attack by reducing the preference for, or effect of, herbivores on the damaged plant. Active responses involve de novo synthesis or energetically costly enzymatic processes, whereas passive responses involve only the consequences of tissue removal. Our study displayed protective enzyme (CAT, POD and SOD) activities in *B. javanica* evidently altered after *C. cinerea* damaged (Fig. 1; Table 1), which was in agreement with a previous report (Golan *et al*., 2013). The result further proved protective enzyme to be involved in the defense against *C. cinerea*. But we didn't test whether protective enzyme in *B. javanica* can effectively deter insect feeding. Furthermore, we observed defoliation phenomenon of *B. javanica* after *C. cinerea* damaged in the field. In our opinion, changes in plant chemistry following defoliation may result from a passive rearrangement of resources within the plant, while enzymatic activation of precursors and synthesis of phytoalexins and proteinase inhibitors is active processes.

Plants have evolved an arsenal of defenses that they have been consumed by herbivores, whose responses are known to occur within several hours after feeding. In this study, CAT, POD and SOD in *C. cinerea* were significantly altered by the foliage maturity (except for CAT), feeding duration and their interaction (Fig. 1D, E and F; Table 2) after feeding for several hours, especially for those on mature leaves. We postulated that *C. cinerea* nymphs preferred to mature leaves of *B. javanica*, for which could attribute to much nutritional components and little secondary metabolites (see supplementary materials). Disappointedly, what responses component of damage signals rapidly induced is still unknown. Currently, we only recognize that plants can actively perceive damage and induce responses to reduce herbivore and its effects on plant fitness though some plant defensive traits are constitutively expressed (Johnson, 2011). Previous work showed that herbivorous insects had also developed numerousdefense mechanisms against free oxygen radicals in response to the oxidative burst in plants. However, little is known about the antioxidant system in Homoptera.

The evolution of such plant-insect association is believed to be driven in part by the insects' need to avoid competition and to escape from enemies (Schoonhoven *et al*., 1998). This process has involved adaptation to the physical and chemical defenses that plants have developed against generalist herbivores. The GST in insects is a multifunctional enzyme involved in the detoxification of xenobiotic and endobiotic compounds through conjugating tripeptide (y-Glu-Cys-Gly)

glutathione (GSH) to hydrophobic substrates. Results from the current study elicited that GST activity in *C. cinerea* gradually enhanced following feeding duration though its level is still lower than control (Fig. 2A; Table 2). The higher level of GST in the control group may be due to starvation stress for 3 h, because the same phenomenon was observed in other invertebrate (Wilczek *et al*., 2013). Furthermore, GST activity in *C. cinerea* feeding on mature leaves was always higher than those on the tender leaves (Fig. 2A). We considered that it needs much more GST to detoxicate these secondary metabolites due to the higher feeding amount.

The AMY is the most ubiquitous polysaccharidase in insects, which digest starch to maltose, followed by a digestion via a glucosidase to glucose (Weidlich *et al*., 2013). Level of AMY in *C. cinerea* reared with mature leaves of *B. javanica* sharply increase. However, AMY in nymphs reared with tender leaves suddenly decreased after feeding for 2 h (Fig. 2B). We ascribed the cause to host preference the same as mentioned above, because AMY content needs rise to digest much more food intake.

Conclusion: our work indicated that they had been coevolved to an adaptation against each other. However, what the plant selection mechanism used by *C. cinerea*, what responses component of damage signals rapidly induced *B. javanica*, and what responses component of secondary metabolites induced *C. cinerea* are still unknown. The answer to these issues will help to understand the mechanism of adaptive evolution of their interaction.

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REFERENCES

Beauchamp, C. and I. Fridowich (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44:276–287.

Cassells, A. C. and R. F. Curry (2001). Oxidative stress and physiological, epigenetic and genetic variability in plant tissue culture: implications for micropropagators and genetic engineers. Plant Cell Tiss. Org. 64:145–157.

Chen, J. J., L. Wang, Z. Y. Chen, D. W. Li, L. F. Zheng and Y. Z. Li (2012). Spatial distribution pattern sampling technology of *Coloana cinerea* Dworakowska. Biol. Disaster Sci. 35:111–112.

de Vos, M., J. H. Kim and G. Jander (2007). Biochemistry and molecular biology of Arabidopsis-aphid interactions. Bio Essays 29:871–883

Golan, K., K. Rubinowska and E. Górska-Drabik (2013). Physiological and biochemical responses of *Fern nephrolepis* Biserrata (Sw.) Schott. To *Coccus hesperidum* L. infestation. Acta Biol. Cracov. Bot. 55:93–98.

Habig, W. H., M. J. Pabst and W. B. Jakoby (1974). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249:7130–7139.

Johnson, M. T. J (2011). Evolutionary ecology of plant defences against herbivores. Funct. Ecol. 25:305–311.

Li, D. W., Z. Y. Chen, L. Wang, Y. Z. Li, C. Zhang and C. S. Qin (2012). Study on the biological characteristics of *Coloana cinerea* Dworakowska from *Bischofia javanica*. J. Zhongkai Univ. Agri. Eng. 25:24–28.

Radhakissnan, T. M. and P. S. Sarma (1963). Intracellular localization and biosynthesis of catalase in liver tissues. Curr. Sci. 32:1749

Sadasivam, S. and A. Manickam (1996). Biochemical Methods, 2nd Ed. New Age International Publishers, New Delhi (India). 291p.

Schoonhoven, L. M., T. Jermy and J. J. A. van Loon (1998). Insect-plant biology: from physiology to evolution. Chapman and Hall, London (UK). 409p.

Scott, J. G., N. N. Liu and Z. M. Wen (1998). Insect cytochromes P450: diversity, insecticide resistance and tolerance to plant toxins. Comp. Biochem. Physiol. C 121:147–155.

Sun, Y. J (2010). Interaction between *Coloana cinerea* Dworakowska and *Bischofia javanica* Blume and the sensitivity of *C. cinerea* to three insecticides. M.Sc. thesis (unpublished). College of Forestry, Guangxi University, Nanning.

Tuomi, J., P. Niemela, E. Haukioja, S. Siren and S. Neuvonen (1984). Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. Oecologia 61:208–210.

Wang, Y., L. W. Oberley and D. W. Murhammer (2001). Antioxidant defense systems of two lepidopteran insect cell lines. Free Radical Bio. Med. 30:1254–1262.

Weidlich, S., S. Müller, K. H. Hoffmann and J. Woodring (2013). Regulation of amylase, cellulase and chitinase secretion in the digestive tract of the two-spotted field cricket, *Gryllus bimaculatus*. Arch. Insect Biochem. 83:69–85.

Wilczek, G., A. Babczyńska and P. Wilczek (2013). Antioxidative responses in females and males of the spider *Xerolycosa nemoralis* (Lycosidae) exposed to natural and anthropogenic stressors. Comp. Biochem. Physiol. C 157:119–131.