Türk. entomol. derg., 2015, 39 (4): 393-400
DOI: http://dx.doi.org/10.16970/ted.04924

ISSN 1010-6960

Orijinal araştırma (Original article)

Effect of a gall-inducing psylloid, *Pseudophacopteron alstonium* Yang et Li (Hemiptera: Phacopteronidae) on defensive chemistry of *Alstonia scholaris* (L.) R. Br. (Gentianales: Apocynaceae)

Gal yapan bir psylloid *Pseudophacopteron alstonium* Yang et Li (Hemiptera: Phacopteronidae)'un, *Alstonia scholaris* (L.) R. Br. (Gentianales: Apocynaceae)'in kimyasal savunma yapısı üzerine etkisi

Zhen-De YANG[1] Wen-Ling LV[1] Xia-Lin ZHENG[2*]

Shu-Zhong YU[1] Ming LI[1]

Summary

Gall-inducing insect species are capable to escape from defence of host plant species. However, effect of gall-inducing insects on defensive mechanism of host plants is still unclear. The present study was conducted to evaluate the possible chemical changes in the defensive system of *Alstonia scholaris* (L.) R. Br. (Gentianales: Apocynaceae) caused by *Pseudophacopteron alstonium* Yang et Li (Hemiptera: Phacopteronidae) feeding. Total phenolic and tannin, peroxidase, superoxide dismutase, catalase and polyphenol oxidase of gall (G) and non-galled (NG) tissues in one leaf, whole leaf with gall (WG) from 1st to 5th stages induced by *P. alstonium*, and un-galled leaves (UL) were measured in laboratory. High levels of secondary metabolites (i.e., total phenolic and tannin) and protective enzymes were detected, which increased sharply during 1st to 3rd stages of galls and subsequently decreased when nymph stoped feeding or entered into mature (4th or 5th) stages. The recorded high levels of secondary metabolites and protective enzymes in *A. scholaris* could be result of defensive response against *P. alstonium*. The current findings could be helpful for understanding the interaction between plants and gall-formed insects.

Keywords: *Pseudophacopteron alstonium*; *Alstonia scholaris*; gall-inducing insect; defensive response

Özet

Gal yapan böcekler konukçu bitkilerin savunma mekanizmalarından kaçabilme yeteneklerine sahiptirler. Bununla birlikte gal yapan böceklerin bitkilerin savunma mekanizmalarına etkileri hala tam olarak bilinmemektedir. Bu çalışma *Pseudophacopteron alstonium* Yang et Li (Hemiptera: Phacopteronidae)'nin *Alstonia scholaris* (L.) R. Br. (Gentianales: Apocynaceae) üzerinde beslenmesi ile bitki savunma sisteminde meydana gelen kimyasal değişimleri belirlemek için yapılmıştır. *P. alstonium* tarafından birden beşinci seviyeye kadar uyarılmış Gall, aynı yaprakta galsiz yaprak parçası, ve hiç galsiz yapraktaki toplam tannin, peroksidaz, superoksid, dismutaz, katalaz ve polyphenol oksidaz düzeyleri laboratuvar koşullarında ölçülmüştür. Galin birinci evresinden üçüncü evresine kadar bitkide ikincil metabolitlerin (toplam phenolic ve tanin gibi) ve koruyucu enzimlerinde önemli bir artış tespit edilmişken, nimflerin beslenmeyi durdurduğu yada ergin hale geçtiği galin dördüncü ve beşinci evresinde bu değerler belirgin şekilde düşük bulunmuştur. *A. scholaris*'in ikincil metabolitlerinin ve koruyucu enzimlerinde meydana gelen bu artışın bitkinin *P. alstonium*'un beslenmesine verdiği tepkiden kaynaklandığı düşünülmektedir. Elde edilen bu bilgilerin bitki ve bitkilerde gallere neden olan böcekler arasındaki ilişkinin belirlenmesine yardımcı olacaktır.

Anahtar sözcükler: *Pseudophacopteron alstonium*; *Alstonia scholaris*; gal yapan böcek; savunma tepkisi

[1] College of Forestry, Guangxi University, Nanning, China
[2] College of Agriculture, Guangxi University, Nanning, China
* Sorumlu yazar (Corresponding author) e-mail: zheng-xia-lin@163.com
Alınış (Received): 06.03.2015 Kabul ediliş (Accepted): 30.12.2015 Çevrimiçi Yayın Tarihi (Published Online): 08.01.2016

Introduction

Numerous microorganisms and arthropods interact intimately with plants and induce specialized structures – named as gall. The galling habit is especially common in more than 13,000 known gall-forming insects species (Raman et al., 2005; Raman, 2012). Gall-inducing habit has evolved independently and multiple times in insects, resulting co-evolution between the plants and the gall-inducing insects leading to evolved strategies to avoid each other's defense systems (Raman et al., 2005).

Alstonia scholaris (L.) R. Br. (Gentianales: Apocynaceae) has been widely used for landscaping projects in Southeast Asia (including some southern provinces in China), Australia and United States (Kaushik et al., 2011). During recent years, this plant was seriously damaged by *Pseudophacopteron alstonium* Yang et Li (Hemiptera: Phacopteronidae) through gall-inducing, which effects the ornamental value, normal growth and development of *A. scholaris* (Yang & Li, 1983; Qin et al., 2010; Zhang et al., 2011; Lv, 2012). *P. alstonium* has seven generations per year in Nanning City, Guangxi Zhuang Autonomous Region, China. Generation overlapped was observed. Spatial distribution of this species was aggregation distribution under natural conditons (Lv, 2012). Development of gall represents an outgrowth which results due to the interaction between gall-inducing insect and host plant. The production material of gall can provide an optimal material to explore the interaction between gall-inducing insect and host plants (Stone & Schönrogge, 2003). However, effect of gall-inducing psylloid on defensive mechanism of host plants is still unclear. Understanding this information is helpful for exploring the co-evolution between gall-inducing insects and host plants.

The aim of present study was to examine the effect of *P.alstonium* on defensive chemistry of *A.scholaris.* Therefore, the contents of total phenolic, tanni, levels of peroxidase, superoxide dismutase, and catalase and polyphenol oxidase were tested in *A.scholaris* during the five developmental stages of *P.alstonium*.

Materials and Methods

Plant materials

The development stages of galls on *A. scholaris* induced by *P. alstonium* (from 1st to 5th) were collected from Nanning City, China (108°28´E, 22°84´N) during 2011 (Figure 1). Gall (G) and non-galled (NG) tissues from the infested leaves were detected using a scalpel and used as first two treatments. Whereas, whole infested leaves (WG) and un-galled leaves (UL) were used as third and fourth treatment, respectively. UL were synchronously collected from the same *A. scholaris* trees. The leaves form (G, NG and WG) having gall density (≤ 5) were chosen to study the effect of gall density on the nutritional contents, components and secondary metabolites of *A. scholaris*. Insects in galls of the G and WG treatments were flushed using a distilled-water jet (Rongyi Experiment Equipment Co. Ltd., Guangxi, China). About 30 g samples of each treatment were obtained from ten *A. scholaris* leaves. All samples were cut into 1 mm fragments and stored in liquid nitrogen prior to analysis.

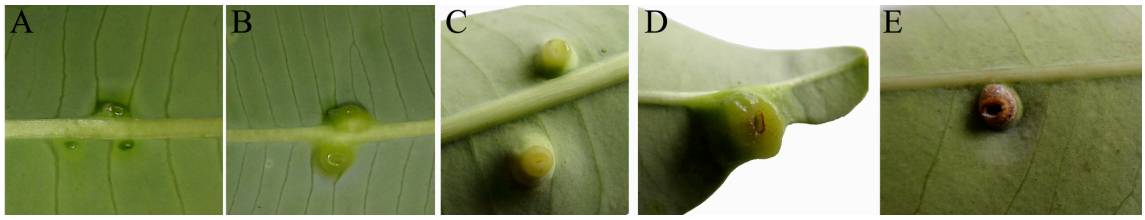

Figure 1. Galls from 1st to 5th developmental stages (A-E) induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*. A. leaf attacked by 1st instar larvae, and a protuberance appeared; B. ostiolate attacked by *P. alstonium* on galls was closed, a green gall formed; C. gall bulged and color changed to aqua or buff, but the color of closed ostiolate showed the amaranth; D. Emergence holes appeared on galls; E. Galls withered after emergence of *P. alstonium* adults.

Determination of total phenolic and tannin contents

Total phenolic and tannin contents in the extracts were determined according to Ainsworth & Gillespie (2007) and Makkar et al. (1993), respectively. Total 1.0 g samples of each treatment was soaked in 150 mL of 50 % ethanol and acetone for 24 h and then filtered to obtain a filtrate. The ethanol and acetone extracts were obtained by evaporating filtrate at room temperature for 3 days. The evaporated extracts were cooled in a desiccator before further analysis. Fifty mg sample of each treatment were added into test tubes containing 2.5 mL of 10% (V/V) Folin-Ciocalteu reagent and 2.0 mL of sodium carbonate (2%, W/V). After shaking, it was left for 2 h and the absorbance was measured at 750 nm using a spectrophotometer (GE Ultrospec 2100 pro, GE Healthcare, UK). Gallic acid was used as standard to obtain a calibration curve (ranging from 0 to 0.8 mg / mL^{-1}). Using the standard curve, the content of total phenolic compounds was calculated and expressed as gallic acid equivalent in milligram per gram (mg/g) of dried extract. As for the level of total tannin, 50 mg of the sample extract was added with 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. Subsequently, 1 mL of 35% sodium carbonate solution and dilute to 10 mL with distilled water was added. Samples were kept at room temperature for 30 min after shaking and the absorbance was read at 725 nm using a spectrophotometer. Blank was established using distilled water instead of the samples. A set of standard solutions of gallic acid was dealt with in the same manner as described above and read against a blank. The results of tannins were expressed in terms of mg gallic acid/g of extract. Sample of each treatment was measured three times.

Assays of peroxidase, superoxide dismutase, catalase and polyphenol oxidase activities

Peroxidase activity was assayed spectrophotometrically with *o*-dianisidine as hydrogen donor (Sadasivam & Manickam, 1996). A 3.5 mL of phosphate buffer (0.2 mol / L NaH_2PO_4 and 0.2 mol / L Na_2HPO_4, pH 6.5) was made in a clean dry cuvette, and 0.2 mL of plant extract and 0.l mL of freshly prepared *o*-dianisidine solution was added. The temperature was maintained from 28 °C to 30 °C and the cuvette was placed in the spectrophotometer set at 430 nm. Then, 0.2 mL of 0.2 M H_2O_2 was added and mixed in solution. Initial absorbance was read at every 30 sec intervals up to 3 min. A graph was plotted with the increase in absorbance against time. From the linear phase, the change in absorbance per min was read. Water blank was used in the assay. Sample of each treatment was measured three times.

The assay of superoxide dismutase activity was carried out with the method of Beauchamp & Fridowich (1971) based on the reduction of nitroblue tetrazolium (NBT) chloride. The reduction of NBT by superoxide radicals to blue coloured formazan was followed at 560 nm. Washed pellets treated with ethanol and chloroform was centrifuged after 15 min at 4 °C and supernatant was used for the assay. The reaction mixture contained, 1.9 mL of phosphate buffer (pH 7.8), 1×10^{-2} M methionine, 6.8×10^{-5} M NBT and 1.17×10^{-6} M riboflavin, with suitably diluted supernatant in a total volume of 3 mL. Illumination of the solution taken in a 10 mL beaker was carried out in an aluminium foil lined box with a 15 W fluorescent lamp for 10 min. Control without the enzyme source was always included. The absorbance was measured at 560 nm using a spectrophotometer. Sample of each treatment was measured three times.

Catalase activity was assayed with the titrimetric method described by Radhakissnan & Sarma (1963). Briefly, 2.5 mL of 0.1 M phosphate buffer, pH 7.5 and 2.5 mL of 0.9% hydrogen peroxide (v/v) in the same buffer were taken and 0.5 mL of the plant extract was added and incubated at room temperature for 3 min. The reaction was then stopped by adding 0.5 mL of 2 N sulphuric acids and the residual hydrogen peroxide was titrated with 0.1 N potassium permanganate solutions. Using similar arrangement, a blank was carried out with boiled enzyme extract. Sample of each treatment was measured three times.

The polyphenol oxidase extraction procedure was described by Ortega-García et al. (2008). In order to establish the extracts of samples from each treatment, frozen samples in liquid nitrogen were pulverized using a pestle and mortar. Powder was homogenized in cold acetone (-20 °C) and polyethyleneglycol (1W: 4V: 1V), and the homogenate refrigerated on dry ice. The solid fraction of the homogenate was separated by vacuum filtration and re-extracted again. The combined acetone extracts were dried overnight. Acetone powder was resuspended in 0.1 M sodium phosphate buffer (1W: 30V), pH 6.8 with 0.3 mg mL^{-1} of type-II trypsin inhibitor, by gently stirring at 4 °C for 30 min before each polyphenol oxidase assay. The sample was filtered through glass wool and the filtrate centrifuged at 10000 × *g* for 20 min at 4 °C. The absorbance was recorded at 475 nm using a spectrophotometer. Sample of each treatment was measured three times.

Statistical analysis

The statistical analysis was performed with SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). Means were compared using a one-way analysis of variance (ANOVA) followed by a Duncan's test ($P < 0.05$).

Results

Variations of total phenolic and tannin contents

Total phenolic content in G was significantly lower than NG, WG and UL treatments ($F = 51.186$; df = 3, 11; $P < 0.001$) at the first stage. However, this result was completely reversed from 2nd to 5th stages. Furthermore, total phenolic contents in G and WG increased from 1st to 4th stages and decreased in the 5th stage (G, $F = 497.578$; df = 4, 14; $P < 0.001$; WG, $F = 100.880$; df = 4, 14; $P < 0.001$). Increasing tendencies of total phenolic contents of both NG and UL suspended at the 4th stage (NG, $F = 430.933$; df = 4, 14; $P < 0.001$; UL, $F = 257.694$; df = 4, 14; $P < 0.001$, Table 1).

Table 1. Total phenolic contents (mg/g) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	21.64±0.43cE	24.56±0.34bC	35.23±0.52cA	33.86±0.56dB	23.25±0.15dD
NG	24.56±0.60aD	30.01±0.99aBC	39.37±0.57bA	30.70±0.85cB	27.81±0.77cC
WG	23.42±0.43bC	29.70±0.46aB	44.82±1.89aA	45.19±0.42bA	31.15±0.13bB
G	19.88±0.60dE	28.82±0.26aD	48.48±0.78aB	50.47±0.64aA	40.13±0.14aC

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Total tannin content in G was lower than UL, NG and WG at the 1st stage ($F = 0.385$; df = 3, 11; $P = 0.767$). From 2nd to 5th stages, total tannin contents in G were always higher than other treatments (2nd, $F = 194.028$; df = 3, 11; $P < 0.001$; 3rd, $F = 367.635$; df = 3, 11; $P < 0.001$; 4th, $F = 373.365$; df = 3, 11; $P < 0.001$; 5th, $F = 144.698$; df = 3, 11; $P < 0.001$, Table 2). Furthermore, the increasing tendency was found in the treatments of UL, NG and WG as the growth and development of galls (UL, $F = 34.759$; df = 4, 14; $P < 0.001$; NG, $F = 58.38$; df = 4, 14; $P < 0.001$; WG, $F = 266.146$; df = 4, 14; $P < 0.001$). As for the treatment of G, increasing tendencies suspended at the 5th stage (G, $F = 355.704$; df = 4, 14; $P < 0.001$).

Table 2. Total tannin contents (mg/g) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	10.03±0.60aC	13.27±0.44bB	13.79±1.00dB	14.83±0.75dB	22.04±0.84cA
NG	10.35±0.50aD	13.19±0.44bC	18.79±0.45cB	18.65±0.70cB	21.64±0.83cA
WG	10.83±0.66aC	12.44±0.36bC	34.52±0.71bB	32.89±0.94bB	40.25±1.81bA
G	9.95±0.77aE	25.22±0.51aD	52.22±1.23aB	58.24±1.48aA	44.16±0.88aC

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Variations of peroxidase, superoxide dismutase, catalase and polyphenol oxidase contents

Peroxidase activities in G were higher than NG, WG and UL treatments from 1st to 3rd stages (1st, $F = 493.252$; df = 3, 11; $P < 0.001$; 2nd, $F = 1044.000$; df = 3, 11; $P < 0.001$; 3rd, $F = 164.921$; df = 3, 11; $P < 0.001$). Furthermore, peroxidase activities ascended in the first two stages and subsequently descended in the four treatments were found (UL, $F = 245.421$; df = 4, 14; $P < 0.001$; NG, $F = 453.697$; df = 4, 14; $P < 0.001$; WG, $F = 617.173$; df = 4, 14; $P < 0.001$; G, $F = 2856.000$; df = 4, 14; $P < 0.001$, Table 3).

Table 3. Peroxidase contents (μg/(g•min)) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	234.24±2.09dA	232.44±6.30dA	144.94±4.64cB	115.95±3.66aC	102.38±1.68aD
NG	270.96±3.51cB	299.38±4.07cA	165.40±5.69cC	111.13±3.77aD	97.80±4.22aD
WG	369.24±4.35bB	460.20±3.82bA	256.73±12.94bC	89.27±3.60bD	99.56±1.58aD
G	408.93±4.33aB	560.31±3.83aA	362.71±4.35aC	105.73±3.81aD	93.75±2.19aE

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Superoxide dismutase activities in G at the first stage were significantly higher than NG, WG and UL treatments ($F = 21.586$; df = 3, 11; $P < 0.001$). However, its activities in G was lower than NG at the 3rd stage ($F = 150.068$; df = 3, 11; $P < 0.001$), NG, WG and UL at the 4th stage ($F = 28.034$; df = 3, 11; $P < 0.001$), and NG and UL at the 5th stage ($F = 61.795$; df = 3, 11; $P < 0.001$). As for the dynamic variations of the four treatments, there was no visible tendency in NG, WG and UL treatments, except for G in which superoxide dismutase activities increased from 1st to 3rd stages and suspended at the 4th stage ($F = 99.06$; df = 4, 14; $P < 0.001$, Table 4).

From 1st to 4th stages, catalase activities in G were higher than other treatments all the time (1st, $F = 18.629$; df = 3, 11; $P < 0.01$; 2nd, $F = 76.314$; df = 3, 11; $P < 0.001$; 3rd, $F = 35.975$; df = 3, 11; $P < 0.001$; 4th, $F = 44.124$; df = 3, 11; $P < 0.001$, Table 5). However, its activity was significantly lower than NG, WG and UL treatments at the 5th stage ($F = 194.357$; df = 3, 11; $P < 0.001$). Dynamic variations of the four treatments were inconspicuous.

Table 4. Superoxide dismutase contents (U/(g•min)) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	195.50±1.74cD	433.02±6.29cB	351.92±4.18cC	448.42±4.95bA	355.79±1.11aC
NG	221.94±5.14bD	470.46±4.01abB	499.23±7.81aA	512.05±3.11aA	336.53±5.94bC
WG	207.54±5.83cE	454.32±6.03bA	339.08±8.00cC	408.88±7.32cB	288.94±5.48cD
G	241.94±3.25aE	480.78±8.71aA	434.98±2.82bB	377.44±19.81cC	291.60±2.23cD

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Table 5. Catalase contents (U/(g•min)) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	22.35±0.64cC	26.90±1.12cB	25.40±0.25cB	35.68±1.32cA	34.05±0.91bA
NG	24.22±1.02cCD	26.13±0.93cC	22.52±1.19cD	46.19±0.42bA	42.82±1.12aB
WG	26.62±0.57bC	33.40±0.23bB	32.19±1.38bB	45.89±1.92bA	27.64±1.17cC
G	29.64±0.59aD	43.74±0.14aB	36.01±0.91aC	57.54±1.28aA	11.77±0.28dE

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Polyphenol oxidase activities in G were significantly higher than other three treatments at the 1st stage ($F = 8.26$; df = 3, 11; $P < 0.01$), and NG and UL treatments at the 4th stage ($F = 95.336$; df = 3, 11; $P < 0.001$). However, polyphenol oxidase activity in G at the 5th stage was significantly lower than other three treatments ($F = 24.815$; df = 3, 11; $P < 0.001$). Polyphenol oxidase activities in G, NG and WG were increased from 1st to 4th stages and suspended at 5th stage except for UL (UL, $F = 324.093$; df = 4, 14; $P < 0.001$; NG, $F = 328.633$; df = 4, 14; $P < 0.001$; WG, $F = 105.887$; df = 4, 14; $P < 0.001$; G, $F = 101.076$; df = 4, 14; $P < 0.001$, Table 6).

Table 6. Polyphenol oxidase contents (U/(g•min)) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	9.96±0.11bC	10.12±0.25cC	20.49±0.59cB	22.01±0.30cA	22.97±0.38bA
NG	9.88±0.07bE	15.44±0.71bD	24.69±0.71bcC	35.42±0.64bA	31.40±0.56aB
WG	10.52±0.36bD	17.96±0.47aC	37.65±2.24aA	41.52±1.40aA	29.56±0.87aB
G	11.52±0.35aD	14.29±0.25bCD	28.53±1.00bB	41.39±1.04aA	17.58±2.28cC

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Discussion

Plants and insects have evolved strategies to avoid each other's defense systems. To counter the herbivore attack, plants produce specialized morphological structures (e.g., hairs, trichomes, thorns, spines and thicker leaves) (Howe & Schaller, 2008) or secondary metabolites and proteins that have toxic, repellent, and/or antinutitional effects on the herbivores (Fürstenberg-Hägg et al., 2013) or trickery (Ellison & Gotelli, 2001). In the current study, results illustrated that high levels of secondary metabolites (i.e., total phenolic and tannin) and protective enzymes in galls were detected. Furthermore, contents of secondary metabolites and protective enzymes of galls increased sharply from the 1st to 3rd stages of galls and subsequently decreased when nymph stops feeding or enters into a mature stage at the 4th or 5th stages of galls. We considered that these high levels of secondary metabolites and protective enzymes maybe were the defensive response when *A. scholaris* attacked by *P. alstonium*.

How plants defend themselves against attack from herbivores has been the subject of considerable interest over many decades. Production of toxic chemicals (e.g., terpenoids, alkaloids, anthocyanins, phenols and quinones) that either kill or retard the development of the herbivores (Hanley et al., 2007) and proteins that have toxic, repellent, and/or antinutitional effects on the herbivores (War et al., 2011) are the direct defenses mediated by plant characteristics. However, gall-formed insects have evolved ways to hijack plant defenses to their own benefit, by sequestering these chemicals and using them to protect themselves from predators, parasitoids, pathogens and other herbivores. This notion has been termed the enemy hypothesis (Price et al., 1987; Schultz, 1992; Hartley, 1998). Our findings showed that levels of secondary metabolites (i.e., total phenolic and tannin) in G were significantly greater than NG, WG and UL treatments, meanwhile the increasing tendencies suspended when nymph stopped feeding or entered into a mature stage. This result is consistent with previous conclusion studied on the galls induced by *Slavum wertheimae* H.R.L. (Hemiptera: Pemphigidae) on *Pistacia atlantica* Desf. (Sapindales: Anacardiaceae) (Rostás et al., 2013). Therefore, we speculated that high levels of gall secondary metabolites confer protection against natural enemies but such a trade-off needs to be investigated.

One of the crucial aspects of host plant resistance against herbivore is the disruption of insect's nutrition. The enzymes that impair the nutrient uptake by insects through the formation of electrophiles includes peroxidase, polyphenol oxidase, superoxide dismutase, etc. (War et al., 2012). In this study, data demonstrated that peroxidase, superoxide dismutase, catalase and polyphenol oxidase activities in G was the highest and gradually enhanced from 1st to 3rd stages of galls (Tables 3-6). However, activities of these enzymes decreased when nymph stops feeding or enters into a mature stage at the 4th or 5th stages of galls (Tables 3-6). These results strongly suggest that higher levels of peroxidase, superoxide dismutase, catalase and polyphenol oxidase in G were the defensive response when *A. scholaris* attacked by *P. alstonium*.

It can be hypothesized that a great deal of secondary metabolites and protective enzymes accumulated in galls when *A. scholaris* leaves damaged by nymph of *P. alstonium*. However, it is confused that how does nymph of *P. alstonium* adapt to the extreme conditions in galls (i.e., high levels of secondary metabolites and protective enzymes) needs to further study.

Acknowledgements

This work was supported by the National Natural Science Foundation of China under Grant (31160165, 31300549, 31560212), Guangxi Natural Science Foundation under Grant (2013GXNSFBA019111), Bossco Scholar Programme on Cultivation of Creative Talents from Young Teachers in Guangxi University, and Guangxi Special Invited Scientist Program in Agric-Environment and Agric-Products Safety (2013B015).

References

Ainsworth, E.A. & K.M. Gillespie, 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature Protocols, 2: 875–877.

Beauchamp, C. & I. Fridowich, 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44: 276–287.

Ellison, A.M. & N.J. Gotelli, 2001. Evolutionary ecology of carnivorous plants. Trends in Ecology & Evolution, 16: 623–629.

Fürstenberg-Hägg, J., M. Zagrobelny & S. Bak, 2013. Plant defense against insect herbivores. International Journal of Molecular Sciences, 14: 10242–10297.

Hanley, M.E., B.B. Lamont, M.M. Fairbanks & C.M. Rafferty, 2007. Plant structural traits and their role in antiherbivore defense. Perspectives in Plant Ecology Evolution and Systematics, 8: 157–178.

Hartley, S.E., 1998. The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall former? Oecologia, 113: 492–501.

Howe, G.A. & A. Schaller, 2008. "Direct Defenses in Plants and Their Induction by Wounding and Insect Herbivores. 7-29". In: Induced Plant Resistance to Herbivory (Ed: A. Schaller) Berlin, Germany: Springer-Verlag, 480 pp.

Kaushik, P., D. Kaushik, N. Sharma & A.C. Rana, 2011. *Alstonia scholaris*: It's phytochemistry and pharmacology. Chronicles of Young Scientists, 2: 71–78.

Lv, W.L., 2012. Biological characteristics of *Pseudophacopteron alstonium* (Homoptera: Phacopteronidae) and its effect on physiological and biochemical indices of *Alstonia scholaris* (Gentianales: Apocynaceae). (Unpublished) Master thesis, Guangxi University, Nanning, China, 69 pp.

Makkar, H.P.S, M. Bluemmel, N.K. Borowy & K. Becker, 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. Journal of the Science of Food and Agriculture, 61: 161–165.

Ortega-García, F., S. Blanco, M.A. Peinado & J. Peragón, 2008. Polyphenol oxidase and its relationship with oleuropein concentration in fruits and leaves of olive (*Olea europaea*) cv. 'Picual' trees during fruit ripening. Tree Physiology, 28: 45–54.

Price, P.W., G.W. Fernandes & G.L. Waring, 1987. Adaptive nature of insect galls. Environmental Entomology, 16: 15–24.

Qin, J.P., Z.Q. Zhang, Z.D. Yang, Y.W. Ju, W.Y. Zhang, W.L. Lv & Y.H. Yu, 2010. Morphological and occurring characteristics of *Pseudophacopteron alstonium*. China Plant Protection, 30: 27–29.

Radhakissnan, T.M. & P.S. Sarma, 1963. Intracellular localization and biosynthesis of catalase in liver tissues. Current Science, 32: 1749.

Raman, A., C.W. Schaefer & T.M. Withers, 2005. Biology, ecology, and evolution of gall-induced arthropods (Vol. 1 & 2). Hampshire, England: Science Publisher Ltd.

Raman, A., 2012. Gall induction by hemipteroid insects. Journal of Plant Interactions, 7: 29–44.

Rostás, M., D. Maag, M. Ikegami & M. Inbar, 2013. Gall volatiles defend aphids against a browsing mammal. BMC Evolutionary Biology, 13: 193.

Sadasivam, S. & A. Manickam, 1996. Methods in Biochemistry. New Age International Private Limited, New Delhi, India, 15 pp.

Schultz, B.B., 1992. Insect herbivores as potential causes of mortality and adaptation in gall forming insects. Oecologia, 90: 297–299.

Stone, G.N. & K. Schönrogge, 2003. The adaptive significance of insect gall morphology. Trends in Ecology & Evolution, 18: 512–514.

War, A.R., M.G. Paulraj, T. Ahmad, A.A. Buhroo, B. Hussain, S. Ignacimuthu & H.C. Sharma, 2012. Mechanisms of plant defense against insect herbivores. Plant Signal Behavior, 7: 1306–1320.

War, A.R., M.G. Paulraj, M.Y. War & S. Ignacimuthu, 2011. Herbivore- and elicitor-induced resistance in groundnut to Asian armyworm, *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Plant Signal Behavior, 6: 1769–1777.

Yang, C.K. & F.S. Li, 1983. A preliminary study on Chinese *Pseudophacopteron* with descriptions of three new species (Homoptera: Psyllidae). Wuyi Science, 3: 120–128.

Zhang, W.Y., W.L. Lv, Z.D. Yang, Z.Q. Zhang & S.M. Lei, 2011. Effect of *Pseudophacopteron alstonium* on photosynthetic parameters and chlorophyll content of *Alstonia scholaris*. China Plant Protection, 31: 7–10.