A Comparative Study on the Susceptibility of Two Species and a Variety of Erythrina to Erythrina Gall Wasp (Quadrastichus erythrinae K.)

Josephine Espinoza Tondo M.S. and Abercio Rotor Ph.D

Abstract—The study characterized gall infestation of the two species and a variety of Erythrina trees by a tiny wasp, Quadrastichus erythrinae Kim, which lives in the soft tissues of young leaves and petioles, stimulating gall formation leading to defoliation, dieback and death. All of the species and a variety studied were susceptible to the pest, with Erythrina variegata var orientalis as the most susceptible, followed by Erythrina variegata and Erythrina crista-galli as the least susceptible. Average gall size in E.variegata var orientalis is 10.27mm on petiole and 5.84mm on the leaf; E. variegata, 9.87mm and 5.81mm respectively; and in E. crista-galli, 5.72 mm and 3.34 mm respectively. All four test plants of E.variegata var orientalis succumbed to the pest after 90 days; while E. variegata remained heavily infested but alive to the end of the experiment. Two of the four test plants of E. crista-galli showed light infestation, while the other two were not infested. The egg, larva and pupa stages of Q. erythrinae K. are spent inside the gall, ensconced in a chamber as the gall increases progressively in size or coalesce with adjacent galls. Phytochemical analyses showed no differences among infested species and a variety of Erythrina.

Keywords—Erythrina, gall, phytochemical analyses, Quadrastichus erythrinae

I. INTRODUCTION

Plants are attacked in many ways resulting in injury or damage on the leaves, stems, roots and other parts. A report included Metro Manila, among the regions where the infestation was observed. Earlier, Josephine E. Tondo M.S., with the Department of Biological Sciences in Philippine Normal University, Philippines Abercio Rotor Ph.D., is Full time Professor of the Graduate School of University of Santo Tomas, Philippines there were reported infestations of Erythrina in neighboring countries such as Taiwan, Singapore, Samoa and Hawaii [4].

One hypothesis which is the nutrition hypothesis attributes the cause of galls to nutrition imbalance as a result of feeding by a pest. The second hypothesis maintains that gall tissues protect the gall forming agent from unfavorable abiotic conditions most especially desiccation [5]. A gall or cesidium is the swelling or overgrowth of any part of the plant which is a result of infestation by certain pests. Generally, the growth and development of gall may later coalesce into a larger mass around the point of attack by the parasite [1]. Among the plants that commonly exhibit galls are lilies, evening primrose, chestnut, apple, oak, spruce, pineapple, cypress, ficus, grape, hickory, pecan, cottonwood and ash flower [3].

The specific objectives of the study are as follows: 1. To validate the cause of gall formation and the nature of host-pest relationship of the gall insect and the Erythrina specimens. 2. To compare the resistance and susceptibility among the three specimens of Erythrina to Q. erythrinae K. through gall production and the extent of this growth. 3. To find out through phytochemical analysis of plant tissues the basis of resistance/susceptibility among the Erythrina specimens.

II. MATERIALS AND METHODS

2.1 Identification and Descriptions of Gall Insects

A. Collection of the Gall Wasp

Gall wasps were collected from the following areas of Bulacan particularly in the North Luzon Expressway (Sta. Rita, Bocaue, Meycauayan and Marilao). Collection of the four stages of the insect’s life cycle namely egg, larva, pupa and adult was done by mechanical means such as using insect net, and light trapping.

B. Validation of the Insect

The collected wasp parasites in all stages of development were preserved with ethyl alcohol in test tube. Insect specimens were also mounted in pin and properly labeled and stored in a box. The specimens were submitted for validation to the Philippine National Museum.
2.2 Treatment For The Erythrina Species

The study on comparative susceptibility of the three (3) Erythrina species was conducted in an improvised greenhouse arranged in Random Complete Block Design. All the four test plants were acclimatized for a period of two weeks.

2.3 All Size And Morphology

The development of the galls in each specimen was observed according to the following criteria:

- a. development and growth of the galls;
- b. size and shape of galls on leaves and petioles; and
- c. anatomy of dissected galls vis-à-vis healthy tissues.

2.4 Identification and Validation of Host Trees

Fresh and dried specimens of the plants were submitted to the Philippine National Museum for identification. In preparing the specimen for drying, the specimens were placed on a single sheet or between the folds of a double sheet of paper. Newspaper and cheesecloth were used to dry the specimen. Dried specimens were placed between sheets of white paper. The presser is made of a pair of bamboo frames measuring 50 by 35 cm. Pressure is applied by tying the frames together with the specimens in between. Drying period was one week.

2.5 Improvised Plant Nursery

An improvised nursery measuring 4m x 4m was constructed adjacent to the residence of the researcher in Sto. Rosario, Paombong, Bulacan. Materials used were nylon mosquito net and bamboo for posts and beams. Four (4) healthy plants two (2) meters in height of each of the two species and one variety of Erythrina were introduced and acclimatized in the nursery for a week.

2.6 Cultivation of the Species

Twelve healthy potted plants, four for each kind of Erythrina under study were procured from Karisz Garden and Landscaping and Fernando Torres Garden in Bulacan. The plants were given proper attention to keep them healthy, shielding them from the gall wasp and other pests. After acclimatization for two weeks, the plants were transplanted directly in RCB design. After two weeks the plants were then openly exposed to the gall wasp which was introduced into the nursery from infested stock.

2.7 Observation of the Erythrina specimens

Comparison was made between and among the three kinds of Erythrina under study principally for their reaction to the gall wasp. Morphological and anatomical observations on the galls produced were recorded and analyzed.

2.8 Susceptibility Tests

The test plants in the nursery were given equal treatment in cultivation, weeding and irrigation to enhance normal growth and development. Susceptibility of each plant to the introduced gall wasp was regularly observed and the incipient appearance recorded. Subsequent developments of infestation and gall formation were made weekly thereafter until the end of the experiment which lasted for six months. These developments were the increase in size and number of galls, coalescing of galls, defoliation, die back of stems, yellowing or wilting of plant parts, failure to produce flowers, and deformed structures.

2.9 Field Observation

The study included field observation of standing Erythrina trees along roads and highways, backyards, open fields and parks in Bulacan, Pampanga, Tarlac and Bataan in Region 3; Laguna and Batangas in Region 4. In Metro Manila, Erythrina trees along Macapagal Boulevard, EDSA, Makati City, Paranaque City, Pasig City and Quezon City were similarly studied. The trees were observed according to these following aspects:

- Healthy, infested or lifeless.
- Approximate age of the trees
- Stage/extent of infestation by species/variety of Erythrina

2.10 Testing of Chemical Properties

Phytochemical analysis was conducted on each specimen according to the presence or absence of alkaloid, sterol, flavonoids, saponins, glycosides and tannin. Fresh leaves of infested and non-infested species of E. variegata and E. crista-galli were air-dried for 8 days. Each of the air-dried plant materials was pulverized with the Thomas Miller grinder. Samples from each of the test plants were obtained and weighed. The extracts were screened in the presence of tannins, saponins, sterols, glycosides, triterpenes, alkaloids and flavonoids. Thin layer chromatography using aluminum silicon gel (F254 Merck) plates were used.

2.11 Statistical Analyses and Data Presentation

All statistical analysis was done using SPSS Program version 13.0. Quantitative test parameters were averaged, the means for each data group computed for significant or non-significant differences at 0.05 per cent level by One-Way ANOVA. Aside from One-Way ANOVA, DUNCAN Multiple Range Test was used to compare its similarities and differences among the studied specimens.

III. RESULTS AND DISCUSSION

The study was conducted to determine the cause of gall formation and host-pest relationship of the gall insect to the Erythrina trees as well to compare their resistance, susceptibility and chemical properties of the three Erythrina species.

3.1 Gall Size on Petiole and Leaves

Galls are mainly on the young petioles and leaves. There is no significant difference on the average size of galls of the three Erythrina specimens. E. variegata orientalis has the largest average size of galls on the petioles with 10.27mm, followed by E. variegata with the average gall size on petioles with 9.87 mm, and E. crista-galli 5.72 mm.
TABLE I
AVERAGE GALL SIZE ON PETIOLES

<table>
<thead>
<tr>
<th>Species</th>
<th>Count</th>
<th>Sum</th>
<th>Average Size of Gall in Petiole</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. variegata</em> var orientalis</td>
<td>56</td>
<td>575.17</td>
<td>10.27089</td>
<td>15.84157</td>
</tr>
<tr>
<td><em>E. variegata</em></td>
<td>81</td>
<td>799.20</td>
<td>9.867778</td>
<td>10.16739</td>
</tr>
<tr>
<td><em>E. crista-galli</em></td>
<td>5</td>
<td>20.61</td>
<td>5.722</td>
<td>0.72622</td>
</tr>
</tbody>
</table>

ANOVA
Source of Variance  | SS    | Df  | MS    | F     | P-value | F-crit |
Between Group       | 95.01348 | 2   | 47.50674 | 3.789454 | 0.024978 | 3.061234 |
Within Group        | 174.583  | 139 | 12.53657 |        |        |        |
Total               | 269.59648 | 141 |        |        |        |        |

Fig. 1. Average Size of Galls on Petiole

There is a significant difference among the specimens on the average size of galls on the leaves, *E. variegata* orientalis the highest (5.84 mm), followed by *E variegata* orientalis (5.81 mm) and *E crista-galli*, the least with 3.34 mm.

TABLE II
AVERAGE GALL SIZE ON LEAVES

<table>
<thead>
<tr>
<th>Species</th>
<th>Count</th>
<th>Sum</th>
<th>Average Size of Gall in Petiole</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Erythrina variegata</em> var orientalis</td>
<td>54</td>
<td>313.50</td>
<td>5.84</td>
<td>6.805563</td>
</tr>
<tr>
<td><em>Erythrina variegata</em></td>
<td>84</td>
<td>401.27</td>
<td>5.81</td>
<td>2.310213</td>
</tr>
<tr>
<td><em>Erythrina crista-galli</em></td>
<td>15</td>
<td>50.11</td>
<td>3.34</td>
<td>2.129707</td>
</tr>
</tbody>
</table>

ANOVA
Source of Variance  | SS    | df  | MS    | F     | P-value | F-crit |
Between Group       | 84.05094 | 2   | 42.02547 | 10.82364 | 0.0000406 | 3.053360 |
Within Group        | 582.4125  | 150 | 3.88275  |        |        |        |
Total               | 666.46344 | 152 |        |        |        |        |

To validate the homogeneous characteristics, Duncan Multiple Range Test was used. DMRT showed the relationship of the three specimens *E. variegata orientalis* and *E variegata* exhibited homogeneous characteristics with the significant value of 0.772. This is in contrast with *E crista-galli* which has a significant value of 1.00.

TABLE III
DMRT GALLS ON PETIOLES

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duncan a,b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. crista-galli</em></td>
<td>5</td>
<td>5.7220</td>
<td>9.8878</td>
</tr>
<tr>
<td><em>E. variegata</em></td>
<td>81</td>
<td>10.2709</td>
<td></td>
</tr>
<tr>
<td><em>E. variegata</em> var orientalis</td>
<td>56</td>
<td></td>
<td>1.0000</td>
</tr>
<tr>
<td>Sig.</td>
<td></td>
<td>0.7723</td>
<td></td>
</tr>
</tbody>
</table>

For the gall size of leaves among the three specimens, *E variegata* and *E variegata* orientalis exhibited homogeneous characteristics with a significant value of 0.935. This is contrast to the *Erythrina crista-galli* which has a significant value of 1.00.

TABLE IV
DMRT GALLS ON LEAVES

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duncan a,b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. crista-galli</em></td>
<td>15</td>
<td>3.3407</td>
<td>5.8465</td>
</tr>
<tr>
<td><em>E. variegata</em></td>
<td>64</td>
<td>5.8072</td>
<td></td>
</tr>
<tr>
<td><em>E. variegata</em> var orientalis</td>
<td>54</td>
<td></td>
<td>0.935</td>
</tr>
<tr>
<td>Sig.</td>
<td></td>
<td>1.0000</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Nature and Extent of Damage by EGW

A. External characteristics of the gall.

The gall wasp caused heavy infestation particularly on *E variegata orientalis* and *E. variegata*. In the greenhouse, infestation was observed two weeks after inoculation. All test
plants of *E. variegata orientalis* succumbed to the pest after ninety days. All test plants of *E. variegata* were heavily infested but managed to remain alive to the end of the experiment. For *E. crista-galli*, two of the four test plants were slightly infested, but succumbed to the pest nonetheless; the other two survived. Newly formed galls (green color) measured 3.01mm and increased to 20.33mm upon maturity (brownish). Matured galls have “craters” which are actually exit holes for the emerging adult gall wasp. The holes remain open, and dry up contributing to the subsequent drying of the whole leaf. This is exacerbated by the coalescing of galls on a single leaf which may also include the petiole.

B. Deformation, Wilting, Yellowing and Defoliation of Leaves

Aside from the formation of galls, wilting and yellowing of leaves and consequent defoliation were observed. *E. variegata orientalis* exhibited heavily defoliation followed by *E. variegata*, and least, *E. crista-galli*.

C. Bearing Flowers

*E. variegata orientalis* and *E. variegata* species did not bear flowers, during the whole experimental period. *E. crista-galli* on the other hand produced flowers in the months of December and February.

D. Microscopic Anatomical Observation

The gall wasp attacked the young shoots which actually are made of succulent stems, leaves and petioles. The internal structures of these parts individually dissected, and studied under the compound microscope. These included cross section of the petioles, leaves, and galls at different stage of infestation. The parenchyma cells serve as storage of food and water. They are primary sites of metabolic functions such as photosynthesis, respiration and protein synthesis [7].

E. Cross Section of Leaves of the Three Erythrina species

Based on the observation gathered, parenchyma tissues found in the cross sections of leaves show basic similarity with those in the petioles, *E. variegata orientalis* and *E. variegata* have thicker portions of parenchyma tissues while *Erythrina crista-galli* has a relatively thinner layer of parenchyma tissues. There are collenchyma cell and fibrovascular tissues (dark colored cells) scattered among the parenchyma cells which apparently add to rigidity and resistance to other kinds of damage including pest attack.

F. Internal Characteristics of Galls

The wasp larvae which develop within the plant tissue induce the formation of galls in young leaves and petioles. As the infestation progresses, the leaves curl and appear deformed while petioles and shoots become swollen. After the larval stage the insect pupates within the leaf and stem tissue [2]. Based on observation, upon reaching adult stage the wasp gnaws its way out, thus creating a crater or hole on the gall. The stages of the life cycle of *Q. erythrinae* were spent inside the gall found in the *Erythrina* species. Upon the growth and development of the *Erythrina* gall wasp in the young petioles and leaves of the species, disarranged and non-uniform sizes of parenchyma and other parts of the ground tissues of the infested plants were observed.

Observations showed that the *Erythrina* species particularly *E. variegata* and *E. variegata var orientalis* have more EGW to occupy the area with numerous parenchyma tisssues and at the same time, *E. variegata* and *E. variegata var orientalis* were exhibited dark structures found in the exterior part of the chamber where infested part were observed. In *E. crista-galli*, EGW developed on the peripheral part of the petiole.

Based on observations, developing wasps stayed on more parenchyma tissues where in it grow and develop.

The same observation was made on the cross section of *Erythrina* leaves. Differentiated tissues consist of parenchyma cells that have no uniform structures. It has no uniform sizes of cells. Disarranged structures of cells were found. Clear areas of chambers were observed around the parasitic insect. All the three *Erythrina* species were stressed due to the presence of hard tissue inside the infested areas. *Erythrina* gall wasp occupies the center part of the differentiated tissue of leaves particularly in *E. variegata* and *E. variegata var orientalis* shown in Fig. 1 and 2 while in *E. crista-galli*, the peripheral portion was occupied by the insect pest.

G. Death and Infestation of Three Erythrina Species Inside the Greenhouse

Death and infestation was observed in the two *Erythrina* species and a variety after the transfer of infested collected parts of *E. variegata* inside the greenhouse and after the transfer of *Q. erythrinae*, the development and production of galls were observed. The observed infestation of *E. variegata var orientalis* was started on the third week of September. First species of *E. variegata* var orientalis died on the second week of October in the same year. While the remaining species of *E. variegata var orientalis* completely died during the first week of February. In *E. variegata* species, gall production was observed on the first week of October. Heavy infestations in all *E. variegata* were prominently exhibited in the month of February. Meanwhile, in *E. crista-galli*, gall production was observed on the third week of January. Light infestation was observed in the month of February. Only two *E. crista-galli* were partially infested.

<table>
<thead>
<tr>
<th>Partially infested species</th>
<th>Heavy infested species</th>
<th>Dead species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead species</td>
<td>Partially infested species</td>
<td>Healthy species</td>
</tr>
<tr>
<td>Heavy infested species</td>
<td>Dead species</td>
<td>Heavy infested species</td>
</tr>
<tr>
<td>Dead species</td>
<td>Heavy infested species</td>
<td>Healthy species</td>
</tr>
</tbody>
</table>

IV. FIELD STUDY
Field observation and survey were made in selected areas of Regions 3, 4 and Metro Manila. For Region 3, some parts of Bulacan, Pampanga, Tarlac and Bataan were observed. For the Region 4, selected areas of Laguna and Batangas were studied. In Metro Manila, some places such as Macapagal Boulevard, Pasay City, Paranaque City, Edsa, Pasig City and Quezon City were observed.

A. Effects of EGW on Field Observations

The collected data of healthy and infested from the survey study of healthy, infested and dead Erythrina trees in selected areas of Regions 3 and 4 and parts of Metro Manila. For Region 3, some parts of Bulacan, Pampanga, Tarlac and Bataan were observed. For the Region 4, selected areas of Laguna and Batangas were studied. In Metro Manila, some places such as Macapagal Boulevard, Pasay City, Paranaque City, Edsa, Pasig City and Quezon City were observed.

B. Death and Infestation of the Three Erythrina Species in the Field

Field observation and survey were made in selected areas of Regions 3, 4 and Metro Manila. For Region 3, some parts of Bulacan, Pampanga, Tarlac and Bataan were observed. For the Region 4, selected areas of Laguna and Batangas were studied. In Metro Manila, some places such as Macapagal Boulevard, Pasay City, Paranaque City, Edsa, Pasig City and Quezon City were observed.

C. Field Observations

The data obtained from observing of Erythrina trees in selected areas of Regions 3 and 4 and parts of Metro Manila. As shown in the table above, there were 280 Erythrina trees died. 101 Erythrina trees were totally infested. Bearing of flowers by Erythrina variegata and Erythrina variegata var orientalis trees were not observed during flowering months.

V. VALIDATION OF THE ERYTHRINA GALL WASP

The adult female wasp lays its eggs into the parenchyma cells of differentiated tissues. The eggs hatch within 7 days and the larva starts feeding on the surrounding cells. The larva undergoes moulting enshrouded in a chamber inside the gall eating the parenchyma tissues. As a result, cells multiply apparently triggered by the damage. The larva makes only one chamber where it feeds, grows and pupates. The pupal stage is spent in the same chamber. A dult wasps cement the lining of its chamber with its secretion to make it impervious to water. The adult emerges its way out into the open, leaving a gaping hole on the gall.

VI. PHYTOCHEMICAL ANALYSIS

Phytochemical tests were made on these Erythrina species on sterols, triterpenes, flavonoids, alkaloids, saponin, glycosides and tannins to be able to identify other factors that may contribute in the susceptibility among the species and a variety of Erythrina. Phytochemical analysis showed some similarities and differences on the three Erythrina species tested. Sterols are positive in E.variegata, E.variegata var orientalis and E. crista-galli. Flavonoids are present in E. variegata, E. variegata var orientalis and E. crista-galli. Alkaloid is present in E. variegata, E. variegata var orientalis and E. crista-galli. Tannin is present in E. variegata, E. variegata var orientalis and E. crista-galli. Triterpene is not present in E. variegata, E. variegata var orientalis and E. crista-galli. Saponin is present in E. variegata var orientalis and not present in E. variegata and E. crista-galli. Glycoside is present in E. variegata var orientalis and E. crista-galli, however, it is not present in E. variegata.

VII. CONCLUSION

Erythrina species and variety exhibited variations with respect to their susceptibility. Validation of insect pest and plant species and variety was done in Philippine National Museum. From the results of the experiment, the researcher has arrived at the following conclusions:

1. It is the same gall wasp (Q. erythrinae K.) that insects and causes galls in the two Erythrina species and a variety, thus proving the hypothesis of a single wasp parasite responsible in the formation of galls in the two host species and a variety.
2. Gall size significantly varies in the three species with E. variegata having the biggest (5.54mm) and E. crista-galli the least (3.34 mm), which proves that there is a significant difference between and among the two Erythrina species and a variety as far as the size, morphology of the galls is concerned.
3. Of the two species of Erythrina and a variety, E. crista-galli is resistant, while the other a Erythrina species and a variety are susceptible to the parasitic wasp. There is no significant difference between E. var orientalis and E. variegata on their susceptibility to wasp parasite.
4. There is the difference on the presence of triterpenes found in species and variety of Erythrina. Triterpene is present in non-infested E. crista-galli while not absent in infested and non-infested E.variegata and in infested E.crista-galli.

REFERENCES