



Faculty of Resource Science and Technology

**Oviposition Behaviour and Genetic Relatedness of
Scirpophaga incertulas Populations from Northwestern Sarawak**

Nur Najwa Hamsein

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Oviposition Behaviour and Genetic Relatedness of
Scirpophaga incertulas Populations from Northwestern Sarawak

Nur Najwa Hamsein

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Signature

Name: Nur Najwa Hamsein

Matric No.: 15020876

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date:

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ABSTRACT

The rice yellow stem borer, *Scirpophaga incertulas* (Walker) is an important pest of rice in Sarawak, Malaysian Borneo. For a better management of this pest, it is crucial to understand its biology and also other aspects such as the oviposition behaviour and the genetic structure which is unknown. The observation on the life cycle of *S. incertulas* was carried out under laboratory condition. The incubation period ranged between 7–9 days. The larval duration completed in 38–42 days. The pupal duration ranged 7–10 days. The female moths longevity was longer than the male moths with an average 4.30 ± 0.90 and 3.70 ± 0.50 days respectively. In this study, rearing of *S. incertulas* under laboratory condition seems to be not feasible. Mortality was the highest at larval stages hence resulted in low adult emergence. For the study on *S. incertulas* oviposition behaviour, several aspects of its behaviour on Sarawak rice landraces were observed in a net house experiment. A total of 180 females of *S. incertulas* were given access to six different rice landraces at three plant ages. The results indicated that rice plants at four-month old were strongly preferred for oviposition regardless of landraces. This is probably because of the height of the older plants as compared to the younger plants. Plants with high stature is easier to be identified by flying moths, making it preferable for the moths to rest and oviposit. The vertical distribution of egg masses on plant were varied. Oviposition on leaf surfaces showed a clear preference on the abaxial leaf surface. To analyze the genetic relatedness of *S. incertulas* populations, four ISSR and primers and two RAPD primers were used. The combined ISSR and RAPD DNA fingerprint had 78 reliable bands with 48 polymorphic bands. The analysis of pooled ISSR-RAPD showed *S. incertulas* population from Kuching and Samarahan were highly related with the index 0.72. The genetic diversity among *S. incertulas* populations estimated were comparable and the AMOVA analysis showed higher genetic variation within population.

Higher genetic variation within population may suggest the presence of different biotypes in each population. The present study is the first attempt of assessing the genetic relatedness of *S. incertulas* populations from northwestern Sarawak. In conclusion, the life cycle duration of *S. incertulas* reared under laboratory condition was estimated with an average 60 days. The adult females preferred four-month old plant for oviposition as compared to younger plants. The populations of *S. incertulas* from northwestern Sarawak require further validation by adding more markers to reveal the genetic diversity.

Keywords: *Scirpophaga incertulas*, Sarawak rice landraces, oviposition behaviour, genetic relatedness

***Tingkah Laku Bertelur dan Hubungkait Genetik bagi Scirpophaga incertulas
dari Barat Laut Sarawak***

ABSTRAK

Ulat penebuk batang kuning Scirpophaga incertulas (Walker) adalah perosak penting tanaman padi di Sarawak. Bagi menguruskan perosak ini dengan lebih baik, kefahaman biologi dan juga aspek-aspek lain seperti tingkah laku bertelur dan juga struktur genetik adalah penting. Akan tetapi maklumat tersebut belum diketahui. Pemerhatian terhadap kitaran hidup S. incertulas telah dijalankan di dalam makmal. Tempoh pengeraman serangga ini berjulat 7–9 hari. Jangka masa peringkat larva berjulat 38–42 hari. Jangka masa peringkat pupa berjulat 7–10 hari. Rama-rama betina hidup lebih lama berbanding rama-rama jantan dengan purata masing-masing 4.30 ± 0.90 dan 3.70 ± 0.50 hari. Dalam kajian ini, pemeliharaan S. incertulas di dalam makmal adalah tidak sesuai. Kadar kematian yang tinggi pada peringkat larva menyebabkan kemunculan peringkat dewasa yang rendah. Bagi kajian tingkah laku bertelur, beberapa aspek tingkah laku bertelur pada beberapa jenis “landrace” padi Sarawak telah dijalankan di dalam eksperimen rumah jaring. Sebanyak 180 ekor S. incertulas betina telah dilepaskan ke dalam rumah jaring yang mengandungi enam “landrace” padi pada tiga peringkat umur. Keputusan menunjukkan padi berumur empat bulan adalah pilihan utama untuk bertelur tanpa mengira “landrace” padi. Ini berkemungkinan disebabkan oleh ketinggian pokok empat bulan berbanding dengan pokok yang lebih muda. Pokok padi yang tinggi adalah lebih mudah dikenalpasti oleh rama-rama yang sedang terbang, seterusnya membuatkan pokok tersebut lebih disukai untuk berehat dan bertelur. Taburan kelompok telur pada pokok yang dinilai secara menegak adalah berbeza-beza. Pengeluaran telur pada permukaan daun menunjukkan dengan jelas kesukaan pada permukaan “abaxial”. Bagi menganalisa hubungkait genetik populasi S.

incertulas, empat primer ISSR dan dua RAPD telah digunakan. Data daripada kombinasi ISSR-RAPD menghasilkan 78 jalur dengan 48 jalur adalah polimorfik. Analisa ini juga menunjukkan populasi *S. incertulas* dari Kuching dan Samarahan mempunyai hubungan yang rapat dengan indeks 0.72. Kepelbagaian genetik di antara populasi *S. incertulas* yang dianggarkan daripada data kombinasi ISSR-RAPD adalah setanding manakala analisa AMOVA menunjukkan terdapat variasi genetik yang tinggi di dalam populasi. Variasi genetik yang tinggi di dalam populasi mengesyorkan kehadiran “biotype” berbeza dalam setiap populasi. Ini merupakan percubaan pertama dalam menilai hubungkait genetik *S. incertulas* dari Barat Laut Sarawak. Kesimpulannya, tempoh kitaran hidup *S. incertulas* yang dipelihara di dalam makmal dianggarkan berpurata 60 hari. Rama-rama betina lebih menyukai pokok berumur empat bulan berbanding pokok yang lebih muda. Hubungan genetik antara populasi *S. incertulas* dari barat laut Sarawak memerlukan pengesahan lanjut dengan menambah lebih banyak “marker” untuk memperlihatkan kepelbagaian genetik.

Kata kunci: *Scirpophaga incertulas*, “landrace” padi Sarawak, tingkah laku bertelur, hubungkait genetik

TABLE OF CONTENTS

| | Page |
|-------------------------------------|-------------|
| DECLARATION | i |
| ACKNOWLEDGEMENT | ii |
| ABSTRACT | iii |
| <i>ABSTRAK</i> | v |
| TABLE OF CONTENTS | vii |
| LIST OF TABLES | xiii |
| LIST OF FIGURES | xvi |
| LIST OF ABBREVIATIONS | xix |
| CHAPTER 1: INTRODUCTION | 1 |
| 1.1 Study Background | 1 |
| 1.2 Problem Statements | 4 |
| 1.3 Objectives | 5 |
| CHAPTER 2: LITERATURE REVIEW | 6 |
| 2.1 Sarawak Rice | 6 |

| | | |
|---------|---|----|
| 2.1.1 | Rice Cultivation in Sarawak | 6 |
| 2.1.2 | Traditional Varieties of Rice in Sarawak | 8 |
| 2.1.3 | Rice Production in Sarawak | 10 |
| 2.1.4 | Traditional Rice Characteristics | 10 |
| 2.2 | Pest of Rice | 11 |
| 2.2.1 | The Rice Stem Borer | 11 |
| 2.2.2 | The Rice Yellow Stem Borer, <i>Scirpophaga incertulas</i> (Walker) | 12 |
| 2.2.3 | General Morphology of Rice Yellow Stem Borer <i>Scirpophaga incertulas</i> (Walker) | 13 |
| 2.2.3.1 | Eggs | 13 |
| 2.2.3.2 | Larvae | 13 |
| 2.2.3.3 | Pupae | 14 |
| 2.2.3.4 | Adults | 14 |
| 2.3 | Damages and Crop Losses | 15 |
| 2.4 | Management | 17 |
| 2.4.1 | Biological Control | 18 |

| | | |
|---|--|-----------|
| 2.4.2 | Cultural Control | 18 |
| 2.4.3 | Chemical Control | 19 |
| 2.4.4 | Host Plant Resistance | 20 |
| 2.5 | Type of Resistance | 21 |
| 2.6 | Rice Stem Borer Resistance in Rice Varieties | 22 |
| 2.7 | Preference of <i>S. incertulas</i> for Oviposition | 23 |
| 2.8 | The Molecular Studies | 24 |
| 2.8.1 | Inter Simple Sequence Repeats (ISSR) | 25 |
| 2.8.2 | Random Amplified Polymorphic DNA (RAPD) | 25 |
| 2.9 | Detection and Identification of Insect Pest through Molecular Techniques | 26 |
| CHAPTER 3: MATERIALS AND METHODS | | 27 |
| 3.1 | Life Cycle Observation of <i>Scirpophaga incertulas</i> (Walker) | 27 |
| 3.1.1 | Plant Materials | 28 |
| 3.1.2 | Insects Sampling for Initiation | 28 |
| 3.1.3 | The Rearing of <i>S. incertulas</i> for Life Cycle Observation | 29 |

| | | |
|---------|--|-----------|
| 3.1.3.1 | Observation of Egg Masses and Eggs | 30 |
| 3.1.3.2 | Observation of Larvae | 32 |
| 3.1.3.3 | Observation of Pupae | 33 |
| 3.1.3.4 | Observation of Adults | 33 |
| 3.2 | Oviposition Behaviour of <i>Scirpophaga incertulas</i> (Walker) on Sarawak Rice Landraces | 35 |
| 3.2.1 | Plant Materials | 35 |
| 3.2.2 | Insects | 36 |
| 3.2.3 | Data Collection and Statistical Analysis | 38 |
| 3.3 | Genetic Relatedness of <i>Scirpophaga incertulas</i> Populations from Northwestern Sarawak | 40 |
| 3.3.1 | Sampling | 40 |
| 3.3.2 | DNA Extraction | 41 |
| 3.3.3 | ISSR and RAPD Amplification | 42 |
| 3.3.4 | Data Analysis | 44 |
| | CHAPTER 4: RESULTS | 46 |

| | | |
|-------|--|----|
| 4.1 | Life Cycle Observation of <i>Scirpophaga incertulas</i> (Walker) | 46 |
| 4.1.1 | Observation of Egg Masses and Eggs | 48 |
| 4.1.2 | Observation of Larvae | 49 |
| 4.1.3 | Observation of Pupae | 51 |
| 4.1.4 | Observation of Adults | 52 |
| 4.2 | Oviposition Behaviour of <i>Scirpophaga incertulas</i> (Walker) on Sarawak Rice Landraces | 55 |
| 4.2.1 | Rice Landrace-Plant Age Preference | 55 |
| 4.2.2 | Vertical Distribution of Egg Masses | 57 |
| 4.2.3 | Leaf Surface of Preference for Oviposition | 58 |
| 4.3 | Genetic Relatedness of <i>Scirpophaga incertulas</i> Populations from Northwestern Sarawak | 60 |
| 4.3.1 | Polymorphism of ISSR and RAPD markers | 60 |
| 4.3.2 | Similarity Coefficient | 63 |
| 4.3.3 | Cluster Analysis | 67 |
| 4.3.4 | Genetic Diversity Analysis | 71 |

| | |
|--|-----|
| CHAPTER 5: DISCUSSION | 74 |
| 5.1 Life Cycle of Rice Yellow Stem Borer, <i>Scirpophaga incertulas</i> (Walker) | 74 |
| 5.2 Oviposition Behaviour of <i>Scirpophaga Incertulas</i> (Walker) on Sarawak Rice Landraces | 79 |
| 5.3 Genetic Relatedness of <i>Scirpophaga incertulas</i> Populations from Northwestern Sarawak | 81 |
| CHAPTER 6: CONCLUSION AND RECOMMENDATIONS | 85 |
| 6.1 Conclusion | 85 |
| 6.2 Recommendations for Further Research | 86 |
| REFERENCES | 88 |
| APPENDIX | 107 |

LIST OF TABLES

| | | Page |
|-----------|---|-------------|
| Table 2.1 | Estimated area, yield and production of rice in Sarawak 2018 | 8 |
| Table 2.2 | Popular rice variety according to region | 9 |
| Table 2.3 | Estimated planted area and rice production in Malaysia 2018 | 10 |
| Table 3.1 | The ISSR primers and its annealing temperature | 43 |
| Table 3.2 | The RAPD primers and its annealing temperature | 44 |
| Table 4.1 | Life cycle observation of <i>S. incertulas</i> reared on rice plant under laboratory condition | 47 |
| Table 4.2 | Measurement of egg masses, larvae, pupae and adults of <i>S. incertulas</i> | 54 |
| Table 4.3 | Mean number of egg masses and eggs per plant (\pm SD) laid at one-, two- and four-month-old | 56 |
| Table 4.4 | Mean number of egg masses and eggs per plant (\pm SD) laid on six different rice landraces | 56 |
| Table 4.5 | Mean number of egg masses per plant (\pm SD) at different location (lower, middle, or upper region of plant) regardless of rice landraces and plant ages | 58 |

| | | |
|------------|---|----|
| Table 4.6 | Mean number of egg masses per plant (\pm SD) for the oviposition preference on leaf surfaces regardless of rice landraces and plant ages | 59 |
| Table 4.7 | Performance of each ISSR and RAPD primers tested on <i>S. incertulas</i> | 61 |
| Table 4.8 | Summary statistic of ISSR and RAPD analysis | 62 |
| Table 4.9 | ISSR similarity index for the 5 populations of <i>S. incertulas</i> generated from NTSYS-pc2.2 | 64 |
| Table 4.10 | The percentage of variation for the 5 populations of <i>S. incertulas</i> analysed using ISSR markers | 64 |
| Table 4.11 | RAPD similarity index for the 5 populations of <i>S. incertulas</i> generated from NTSYS-pc2.2 | 65 |
| Table 4.12 | The percentage of variation for the 5 populations of <i>S. incertulas</i> analysed using RAPD markers | 65 |
| Table 4.13 | ISSR-RAPD similarity index for the 5 populations of <i>S. incertulas</i> generated from NTSYS-pc2.2 | 66 |
| Table 4.14 | The percentage of variation for the 5 populations of <i>S. incertulas</i> analysed using ISSR-RAPD markers | 66 |

| | | |
|------------|---|----|
| Table 4.15 | Numbers of observed and effective alleles, estimated Nei's genetic variation, Shanon index, and percentage of polymorphic loci based on ISSR markers | 72 |
| Table 4.16 | Numbers of observed and effective alleles, estimated Nei's genetic variation, Shanon index, and percentage of polymorphic loci based on RAPD markers | 72 |
| Table 4.17 | Numbers of observed and effective alleles, estimated Nei's genetic variation, Shanon index, and percentage of polymorphic loci based on ISSR-RAPD markers | 73 |

LIST OF FIGURES

| | | Page |
|------------|---|-------------|
| Figure 2.1 | Damage caused by <i>S. incertulas</i> . a) Dead heart b) White head c) Dead rice plant | 17 |
| Figure 3.1 | a) Portable oviposition cage b) The net house | 27 |
| Figure 3.2 | Light trap and white sheet | 29 |
| Figure 3.3 | Flow chart to obtain eggs for egg number estimation and rearing for life cycle observation | 31 |
| Figure 3.4 | Flow chart to obtain eggs for observation on hatchability and parasitization | 32 |
| Figure 3.5 | Steps in rearing <i>S. incertulas</i> for life cycle observation following methods by Saxena et al. (1990) with modifications | 34 |
| Figure 3.6 | Rice plant age a) One-, b) Two- c) Four-month-old | 37 |
| Figure 3.7 | Circles with different colours and sizes: Rice landraces and plant growth stages. Star: Release point. | 37 |
| Figure 3.8 | a) The adaxial leaf surface with parallel veins forming many ridges b) The abaxial leaf surface with a protruding ridge on the midrib | 39 |

| | | |
|-------------|---|----|
| Figure 3.9 | Region of the rice plant a) Lower b) Middle c) Upper | 39 |
| Figure 3.10 | Sampling area (Retrieved from https://www.vectorstock.com/royalty-free-vector/administrative-map-sarawak-malaysia) | 40 |
| Figure 4.1 | Life cycle of <i>S. incertulas</i> observed under laboratory condition | 46 |
| Figure 4.2 | Stage of egg masses a) The egg mass b) Creamish colour of early stage of eggs c) Greyish black colour of late stage of eggs | 49 |
| Figure 4.3 | a) Egg parasitoids emerging from egg mass b) The egg parasitoids <i>Telonomus</i> sp. | 49 |
| Figure 4.4 | The larval stages a) Newly hatched larvae b) 10 days old larvae c) Mature larvae | 50 |
| Figure 4.5 | Symptoms of larvae infestation on rice plant a) Fresh frass at the basal part b) Tiny hole at the basal part | 51 |
| Figure 4.6 | Pupal stage a) Early stage b) Late stage | 52 |
| Figure 4.7 | The adult stage of <i>S. incertulas</i> a) Female b) Male | 53 |
| Figure 4.8 | Relationship between plant age and number of egg mass | 57 |
| Figure 4.9 | Eggs laid on different leaf surfaces a) Adaxial b) Abaxial c) Leaf sheath | 59 |
| Figure 4.10 | Association between leaf surfaces and number of egg mass | 60 |

| | | |
|-------------|---|----|
| Figure 4.11 | ISSR-PCR amplification patterns of <i>S. incertulas</i> populations using ISSR Primer 881: 1 Kb and 100 bp ladder. Numbers on the top of the lanes indicate the <i>S. incertulas</i> populations. | 62 |
| Figure 4.12 | RAPD-PCR amplification patterns of <i>S. incertulas</i> populations using RAPD Primer C: 100 bp ladder. Numbers on the top of the lanes indicate the <i>S. incertulas</i> populations. | 63 |
| Figure 4.13 | Dendogram of <i>S. incertulas</i> populations constructed using UPGMA based on bands amplified by ISSR primers (Scale at the bottom is Jaccard's coefficient of similarity) | 68 |
| Figure 4.14 | Dendogram of <i>S. incertulas</i> populations constructed using UPGMA based on bands amplified by RAPD primers (Scale at the bottom is Jaccard's coefficient of similarity) | 69 |
| Figure 4.15 | Dendogram of <i>S. incertulas</i> populations constructed using UPGMA based on bands amplified by combined ISSR-RAPD primers (Scale at the bottom is Jaccard's coefficient of similarity) | 70 |

LIST OF ABBREVIATIONS

| | |
|-------|--|
| AE | Elution buffer |
| AFLP | Amplified fragment length polymorphism |
| AL | Lysis buffer |
| AMOVA | Analysis of molecular variance |
| ANOVA | Analysis of variance |
| ATL | Tissue lysis buffer |
| AW1 | Wash buffer 1 |
| AW2 | Wash buffer 2 |
| bp | Base pairs |
| CABI | Centre for Agriculture and Bioscience International |
| COI | Cytochrome c oxidase subunits I |
| COII | Cytochrome c oxidase subunits II |
| cm | Centimeter |
| DALP | Direct amplified length polymorphism |
| DNA | Deoxyribonucleic acid |
| dNTP | Deoxynucleotide triphosphates |
| DOA | Department of Agriculture |
| E | East |
| EPPO | European and Mediterranean Plant Protection Organization |
| etc | Et cetera |
| ETL | Economic threshold level |
| FAO | Food and Agriculture Organization |

| | |
|-------------------|---|
| g | Gram |
| GI | Geographical indication |
| H | Nei's genetic diversity |
| ha | hectare |
| I | Shannon information index |
| IRRI | International Rice Research Institute |
| ISSR | inter simple sequence repeat |
| Kb | Kilobase pairs |
| kg | Kilogram |
| km | Kilometer |
| K ₂ O | Potassium oxide |
| L | Liter |
| m | Meter |
| MgCl ₂ | Magnesium chloride |
| MgO | Magnesium oxide |
| ml | Milliliter |
| mm | Millimeter |
| mM | MiliMolar |
| Mt | Megatonne |
| MyIPO | Intellectual Property Corporation of Malaysia |
| n | Sample size |
| N | North |
| Na | Observed number of alleles |

| | |
|-------------------------------|---|
| n.d. | No date |
| Ne | Effective number of alleles |
| ng | Nanogram |
| NKEA | National Key Economic Area |
| NTSys-pc | Numerical Taxonomy and Multivariate Analysis System |
| P | Percentage of polymorphic loci |
| PCR | Polymerase Chain Reaction |
| PEMANDU | Performance Management and Delivery Unit |
| p.m. | Post meridiem |
| P ₂ O ₅ | Potassium oxide |
| RAPD | Random amplified polymorphic DNA |
| rpm | Revolution per minute |
| SCAR | Sequence-characterized amplified region |
| SISMARP | Sistem Maklumat Racun Makhluk Perosak |
| SARICON | Sarawak Rice Conference |
| SD | Standard deviation |
| sp. | Species |
| spp. | Several species |
| t | Tonne |
| TAE | Tris Acetate EDTA buffer |
| Taq | <i>Thermus aquaticus</i> DNA polymerase |
| TE | Trace elements |
| UPGMA | Unweighted pair group method with arithmetic mean |

| | |
|----|----------------|
| UV | Ultraviolet |
| V | Volts |
| °C | Degree Celsius |
| ∅ | Diameter |
| μl | Microliter |
| μM | MicroMolar |
| % | Percent |
| < | Less than |