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INTRASPECIFIC LIFE CYCLE AND LARVAL INSTAR VARIATIONS AMONG LOCAL POPULATIONS OF ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee) (Lepidoptera: Crambidea) IN THE PHILIPPINES

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ABSTRACT

Intraspecific life cycle with emphasis on larval instar variations of six local populations of F2 generation of Asian corn borer (ACB), *Ostrinia furnacalis* (Guenee), was studied. The initial populations were collected from at least four towns of the different regional sites and reared for one generation on artificial diet under room temperature and relative humidity. Results showed that the populations in Laguna and Leyte had the longest life cycle and the shortest were observed on Isabela populations. Significant differences in the larval duration of the different local populations of the ACB were observed with those from Leyte having the longest followed by Laguna ACB, while those from Isabela and South Cotabato had the shortest. Most significant of the results was the absence of the sixth instar larval period or the pre-pupal stage for ACB populations in Isabela, Camarines Sur, South Cotabato and Bukidnon. Pupal duration and pupal length significantly differed among the six local populations of ACB. The observed discrepancy was also reported in previous ACB life history studies. The results showed differences in the length of larval period and number of larval instars and this may be attributed to several factors such as maternal age, quality of the egg, the kind of host or diet as reported by a researcher a few years ago. It is also possible that there may be other populations of *Ostrinia* in the country. These significant variations in the life cycle, total larval duration and number of larval instars are interesting research areas to pursue in the future. This very basic knowledge will be important in many aspects of ACB control and management.

Key words: Asian corn borer, *Ostrinia furnacalis* (Guenee), life cycle, larval instar, larval period, pupal period

INTRODUCTION

Asian corn borer (ACB) research in the Philippines started in 1906 when Banks (1906) noted that corn plants were seriously damaged in several farms in Luzon. With the establishment of the University of the Philippines College of

Agriculture (UPCA) in Los Banos in 1908, work on this pest continued as UPCA engaged in corn varietal improvement program. During this time, many cornfields were destroyed in the experimental sites and surrounding regions and this even reached the Visayas regions. After almost a decade, ACB remained a very serious insect pest of corn causing yield losses reaching up to 80% (Sanchez, 1971) and even total crop loss (IPB Annual Report 2000). At present, ACB is still the number one insect pest of open pollinated varieties of corn with the supersweet variety being the most susceptible.

Based on past records, Navarro (1911) was the first to describe the life cycle of the corn borer (*Pyrausta vastatrix*) and the characteristic larval damage on the corn plant. Although incomplete, the document gave a clear picture of the basic nature of the pest. Buligan (1929) conducted an excellent thesis research on the biology of the corn borer (*Pyrausta nubilalis*) that included detailed descriptions and illustrations of the different growth stages of the pest. In the Philippines, other studies on ACB biology were done by Guerrero (1965), Calora et al. (1965), Barrion et al (1980) and Camarao (1976). Studies abroad were done in Thailand by Areekul (1964), in Malaysia by Yunus and Hua (1969) and in Indonesia by Patanakamjorn (1975).

The life cycle of ACB reared under laboratory conditions as observed by different researchers using fresh corn stalks and artificial diet are summarized in Table 1. The ACB population was mainly from Laguna. The total developmental period of the corn borer from egg to adult emergence ranged from 25.59 to 41.47 days, with the study of Camarao (1976) having the shortest and that of Buligan (1929), the longest duration. The discrepancy was noted in the total length of larval period and the number of larval instars. The total larval period ranged from 15.5 to 28.89 days with the shortest and longest periods in the study of Guerrero (1965) and Buligan (1929), respectively. The total number of larval instars ranged from 5 [(Areekul 1964), Yunus & Hua (1969) and Camarao (1976)] to 6 [(Buligan 1929), (Guerrero 1965) and Barrion et al. (1981)]. Even with the use of two kinds of diet or host (corn stalks and artificial medium) the numbers of larval instars were not the same (Table 1). In the study of Gabriel and Camarao (1975), there were only five larval instars but a prepupal stage was observed. The inconsistency is an interesting phenomenon and justifies an in-depth reinvestigation of the life cycle of the pest. Far more interesting was the study of Buligan (1929) with the sixth instar lasting for 11 days. It is possible that one of the discrepancies lies in the basic procedure of how these different authors counted the different instars. Many of the above authors did not describe the specific procedures followed, which is a critical consideration in studying larval characteristics of the insect pest.

This study aimed to determine the life cycle of six local populations of the Asian corn borer, *Ostrinia furnacalis* (Guenee), collected from the different major corn growing areas in the Philippines, then describe and discuss the variations observed in larval instars among them.

MATERIALS AND METHODS

Insect Collection

ACB samples were collected from different sites (Figure 1), namely, Isabela State University (ISU) Experimental Station, ISU, Echague, Isabela; IPB Experimental Station Tranca, Bay, Laguna; Camarines Sur State Agricultural College (CSSAC) Experiment Station, CSSAC, Pili, Camarines Sur; Leyte State University (LSU) Experiment Station, LSU, Visca, Baybay, Leyte; IPB Corn Station, Central Mindanao University, Musuan, Bukidnon and Banga Demonstration Farm, Marbel, Koronadal City, South Cotabato. In each province representing a region, collections were done in at least four different towns. Permits for insect collection and transport of live insect specimens were secured from the Bureau of Plant Industry, Los Baños Station, Los Baños, Laguna, for biosafety purposes.

Table 1. Life cycle data (days) on the Asian corn borer, *Ostrinia furnacalis* (Guenee) under laboratory conditions as reported by several authors including the results in the present study and other relevant information.

STAGES OF DEVELOPMENT	BULIGAN 1929	AREEKUL 1964	GUERRERO 1965	YUNUS & HUA 1969	GABRIEL & CAMARAO 1975	CAMARAO 1976	BARRION ET AL 1981	CAASI-LIT &SAPIN (present study) 2012
Egg: Incubation period (days)	4.33	3.67	3.80	3.0	3.0	3	4.0	4.0
Durations of larval instars (days)								
First	3.55	3.31	1.7	3.0	2.0	3.3	6.0	3.83
Second	3.86	2.99	1.7	3.0	2.0	2.2	2.0	2.64
Third	3.78	3.54	1.8	2.0	2.0	2.1	3.0	2.65
Fourth	3.87	3.91	2.4	4.0	2.0	2.2	2.0	3.37
Fifth	4.64	3.34	2.4	6.0	5.0	5.7	5.0	6.64
Sixth	11.48	-	3.2	-	-	-	2.0	6.58
Pre-pupa					3			
Larval period (days)	28.89	17.09	15.50	18.00	26	15.54	20	25.71
Pupal period (days)	8.03	5.32	6.40	6.00	8.0	7.03	9.50	6.72
Egg hatching to adult emergence (days)	41.47	30.30	26.50	27.00	28.0	25.59	33.50	36.43
Number of generations per year	9	12	12	12	-	13	10	-
OTHER RELEVANT INFORMATION RELATED TO THE ABOVE RESULTS								
Name used	Pyrausta nubilalis	Pyrausta salentialis	Pyrausta salentialis	Ostrinia salentialis	Ostrinia furnacalis	Ostrinia furnacalis	Ostrinia furnacalis	Ostrinia furnacalis
Host/Rearing Medium	Corn stalk	Corn stalk	Corn stalk	Corn stalk	Artificial diet*	artificial diet**	artificial diet**	Artificial diet**
Corn Variety	Native flint	-	Phil hybrid	-	Opaque corn	Opaque corn	Opaque corn	IPB Var 1
Geographical origin	UPCA farm	Thailand	Pili Drive UPLB	Malaysia	Pili Drive UPLB	Pili Drive UPLB	UPLB Laguna	UPLB Laguna

*Modified CIMMYT Diet II (Gabriel and Camarao, 1975). **Artificial diet using the ingredients and following the procedures of Jamornman (1972) and Camarao (1976). – No data. CIMMYT = Centro Internacional de Mejoramiento de Maiz y Trigo.

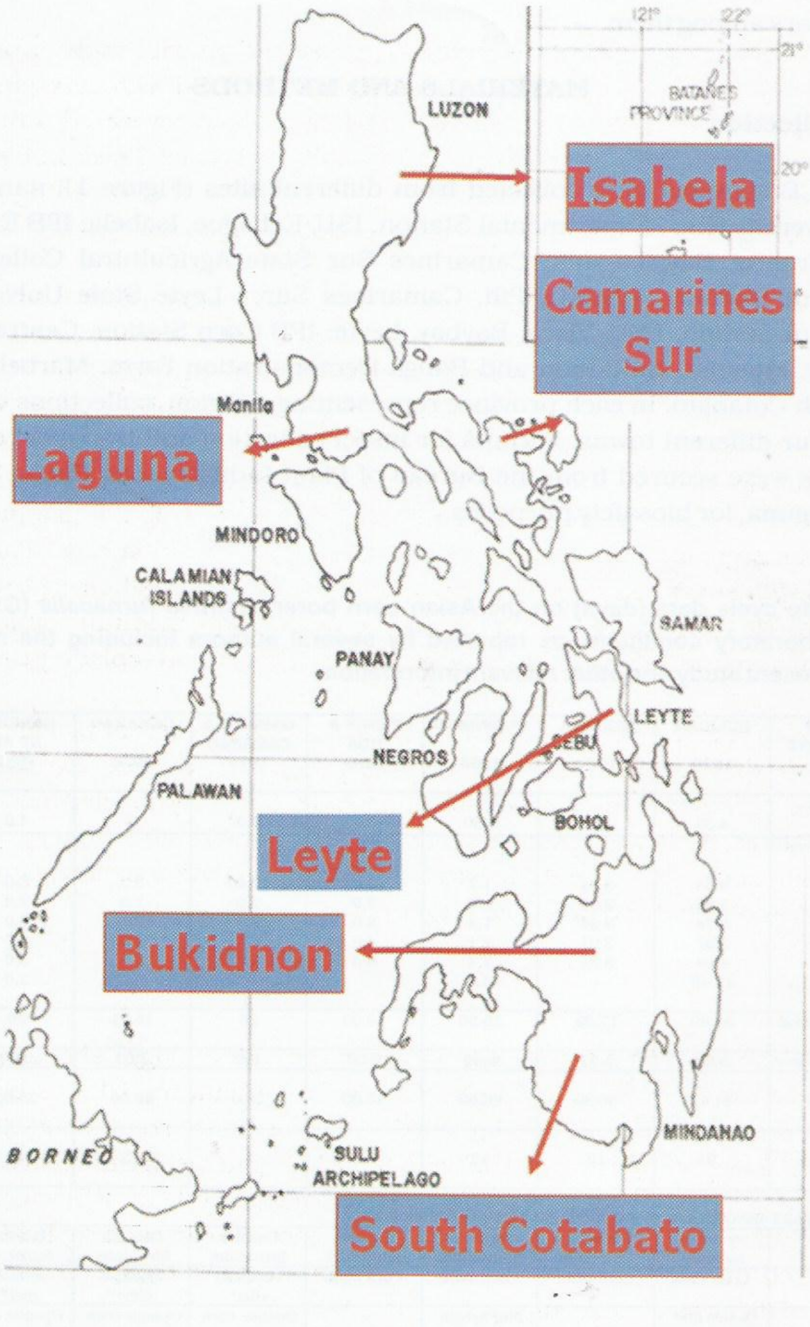

Figure 1. Map of the Philippines showing the six experimental sites where the existing populations of the Asian corn borer [Ostrinia furnacalis (Guenee)] were sampled.

Laboratory Mass Rearing and Handling of Test Specimens

Collected egg masses were placed in a Petri dish with moistened filter paper. The collected larvae were placed in an acrylic pan and fed with corn stalks while the collected pupae were placed in a Petri dish. Later, the larvae were fed with artificial diet and the pupae were transferred to a cage for adult emergence. These initial populations were brought to the Entomology Laboratory, Institute of Plant Breeding, Crop Science Cluster, College of Agriculture, UP Los Baños, College, Laguna.

The artificial diet for the larvae was a modified version of that one developed by Camarao (1976). When the egg masses reached blackhead stage, they were placed in an acrylic pan containing 250 grams artificial diet for the projected 100-125 larvae per pan. This amount of diet was sufficient to complete the whole larval stage of the ACB. Later, the late instar larvae were transferred to an acrylic pan lined with tissue paper. The tissue paper was corrugated and the grooves served as the pupation chamber for the larvae. The pupae were collected and placed in a Petri dish. When the adults were about to emerge, the pupae were transferred to an oviposition cage. The adults were provided with sugar solution. The upper portion of the oviposition cage was lined with a sheet of wax paper, which served as the oviposition medium for female moths. Collection of egg masses was done regularly by retrieving and replacing the wax paper.

To properly manage the ACB populations from different provinces, each ACB population was assigned a separate room. The cages were carefully placed in individual racks and all the acrylic pans were properly labeled according to the collection sites. A net was installed on the doors of each room and the window covered with plastic sheets to prevent the escape of adult ACB. After each experiment, all the adults were collected and preserved in ethyl alcohol.

Life Cycle

All the experiments were conducted in the Entomology Laboratory, Institute of Plant Breeding, Crop Science Cluster, College of Agriculture, UP Los Baños, College, Laguna from October 2006 to May 2007 under ordinary room conditions (25-30°C and 70-80% relative humidity). The field-collected specimens were reared for one cycle and the F2 generation was used in this study. The life cycle of ACB was studied using artificial diet. Three replications were done for each site. In each replication, 50 larvae were reared individually, placing each larva in an opaque cup. The replacement or addition of diet was done regularly.

The larval instar duration or stadium was determined by inspecting each rearing cup and looking for the exuvia or head capsule of each larva. Once located, the exuvia was removed from the container. This procedure was followed to correctly determine the number of instars of the ACB as clearly illustrated in Figure 2. The following parameters were taken: duration of larval instars (days), pupal period (days), duration from egg to adult (days), and length of pupae (mm).

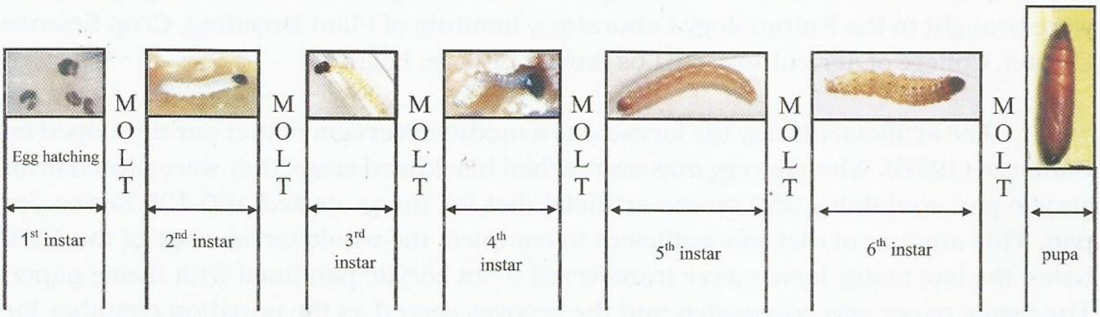

Figure 2. Procedure for determining the different larval instars of the Asian corn borer, *Ostrinia furnacalis* (Guenee), from egg hatching to 6th larval instar.

Statistical analysis

Data were analyzed using one-way ANOVA. Treatment (different ACB populations) means were tested at 5% level of significance using Least Significant Difference (LSD). Welch t-test was used to compare the Laguna and Leyte populations which exhibited six larval instars. Statistical analyses were performed using R Ver. 2.14.1.

RESULTS AND DISCUSSION

Life cycle

The life cycles of the local populations of the Asian corn borer collected from six different regional sites are shown in Table 2. The total developmental period from egg to adult was longest (43 days) for both Laguna and Leyte populations followed by the populations from Bukidnon (40 days). The populations from Camarines Sur and South Cotabato had shorter life cycle (36 days) with the Isabela population having the shortest (35 days). For better comparison, all life cycles were reckoned from egg-laying by adding four days for the incubation period of the egg masses and up to seven days adult life for the average life span of adult ACB.

Table 2. Comparison of the life cycle with emphasis on larval and pupal durations (days) of the F2 Asian corn borer (ACB), *Ostrinia furnacalis* (Guenee), collected from six provinces representing different regional sites and reared on artificial diet under ordinary room conditions (25-30˚C; 70-8O% RH).

Stages of Development	Duration (days)					
	Isabela	Laguna[1]	Camarines Sur	Leyte[1]	South Cotabato	Bukidnon
Larval Instars						
First	2.74 ± 0.02^{d}	3.83 ± 0.02^{a}	3.51 ± 0.40^{b}	$3.62 + 0.05^{b}$	3.32 ± 0.12^{c}	3.26 ± 0.14^{c}
Second	2.02 ± 0.08^{d}	2.64 ± 0.07^{bc}	2.56 ± 0.05^{c}	$2.78 + 0.05^{a}$	2.74 ± 0.12^{ab}	2.66 ± 0.21^{c}
Third	2.54 ± 0.22^{b}	2.65 ± 0.06^{b}	2.92 ± 0.51^{a}	$3.10 + 0.19^{a}$	2.58 ± 0.05^{b}	3.00 ± 0.09^{a}
Fourth	3.16 ± 0.04^{d}	3.37 ± 0.10^{bcd}	3.24 ± 0.16^{cd}	$3.44 + 0.42^{bc}$	3.42 ± 0.23^{b}	3.99 ± 0.16^{a}
Fifth	6.87 ± 0.09^{bc}	6.64 ± 0.49^{b}	6.40 ± 0.28^{c}	$6.70 + 0.44^{bc}$	6.52 ± 0.17^{c}	7.76 ± 0.46^{a}
Sixth[1]	None	6.58 ± 0.49^{b}	None	$6.38 + 0.53^{a}$	None	None
Total Larval Duration[2] (up to fifth larval instar)	17.33 ± 0.10^{c}	19.57 ± 0.25^{b}	18.63 ± 0.34^{c}	19.72 ± 0.25^{b}	18.57 ± 0.18^{c}	20.67 ± 0.23^{a}
Total Larval Duration[3] (up to sixth larval instar)		25.71 ± 0.25		26.02 ± 0.25		
Total Pupal Period	7.27 ± 0.09^{b}	6.72 ± 0.09^{c}	6.77 ± 0.10^{c}	6.66 ± 0.18^{c}	6.75 ± 0.11^{c}	8.34 ± 0.13^{a}
Egg Hatching to Adult Emergence	24.60 ± 0.17^{c}	32.43 ± 1.27^{b}	25.40 ± 0.51^{c}	32.68 ± 3.90^{b}	25.32 ± 0.54^{c}	29.02 ± 0.22^{a}
Egg to Adult	35.00^{c}	43.43^{a}	36.40^{c}	43.68^{a}	36.32^{c}	40.02^{b}

Values (mean $\pm$SD) in the same column, having same letters (per row) are not significantly different at $\alpha = 0.05$ LSD. Number of samples per population = 50 larvae.

[1]ACB populations from Laguna and Leyte exhibited fifth and sixth larval instars.

[2]Total larval duration for population that exhibited five larval instars.

[3]Total larval duration for population that exhibited both fifth and sixth larval instars.

The longer life cycle as shown by the population from Laguna and Leyte is due to the presence of an extra larval instar or stadium. There were individuals from these two populations that underwent five and an extra sixth larval instars. This condition will be explained further in the succeeding section.

Larval duration

Significant differences in larval duration were observed in the different populations of ACB (Table 2). The ACB population from Leyte had the longest larval duration (26.02 days) followed by the ACB population from Laguna (25.71 days). Those from Isabela and South Cotabato had the shortest, 17.33 days and 18.57 days, respectively.

For the first larval instar, the Isabela population had significantly the shortest duration (2.74 days) while the Laguna population had the longest (3.87 days). The longest duration for the second larval instar was recorded in Leyte population followed by the population from South Cotabato. The shortest duration was found in Isabela population. Significant differences were observed in larval stadia for the third, fourth and fifth larval instars and Bukidnon populations showed the significantly longest larval duration. The population from Isabela had the significantly shortest duration for the third and fourth larval instars. Considering only the total larval duration up to fifth larval instar, the population from Bukidnon had the longest larval duration (20.40 days) followed by populations from Leyte (19.72 days) and Laguna (19.57 days). The shortest was observed in Isabela population.

Interestingly, the Laguna and Leyte populations had sixth larval instars lasting 6.58 and 6.38 days, respectively. The presence of 6^{th} larval instar expectedly lengthened the total larval period of both ACB populations.

The ACB from Laguna and Leyte had larvae that underwent five larval instars as well as those that underwent six larval instars. To confirm this phenomenon, data for the length of larval duration and number of larval instars for both populations were reexamined. Those larvae that underwent five larval instars were designated as 'Sub-population A' while those larvae that underwent six larval instars were designated as 'Sub-population B' (Table 3). Comparing 'Sub-population B' of the Laguna and Leyte populations, the different instars differed numerically but not significantly using Welch t-test (Table 3). The proportion of individuals with five and six larval instars was also compared between the Laguna and Leyte populations as shown in Table 4. In the Laguna population, 88.39% and 11.60% of larvae and 97.90% and 2.10% in the Leyte population underwent five and six larval instars, respectively. It is interesting to note the possible occurrence of mixed sub-populations from Laguna and Leyte with the Laguna having higher proportion of larvae that underwent six larval instars.

Table 3. Comparison of the durations (days) of larval instars of the Asian corn borer, *Ostrinia furnacalis* (Guenee), that showed six larval instars (Sub-population B) observed only in Laguna and Leyte populations.

Stages of Development	Laguna	Leyte	t-value
Larva			
1st instar	3.52 ± 0.70	3.99 ± 1.30	-0.7698
2nd instar	2.85 ± 0.69	3.23 ± 0.44	-1.5847
3rd instar	2.66 ± 0.65	2.74 ± 0.41	-0.2862
4th instar	2.77 ± 0.85	3.40 ± 0.91	-y1.4147
5th instar	4.34 ± 1.86	4.60 ± 1.80	-0.2803
6th instar	6.35 ± 2.50	6.60 ± 2.51	-0.201
Total Larval Period	22.49 ± 3.40	24.56 ± 3.26	-1.2665

Mean± SD

Table 4. Proportion of Asian corn borer, *Ostrinia furnacalis* (Guenee), larvae that underwent five (Sub-population A) and six (Sub-population B) larval instars collected from Laguna and Leyte.

Sites	Proportion of ACB larvae (%)	
	Five larval instars (Sub-population A)	Six larval instars (Sub-population B)
Laguna	88.40	11.60
Leyte	97.90	2.10

Previous reports on ACB life history studies [Buligan, 1929; Camarao,1976; Barrion et al (1981)] indicated differences in larval duration as well as the presence or absence of the sixth larval instar or the pre-pupal stage. As shown in Table 1 from the Review of Literature, there is a clear difference in the number of larval instars of ACB. The first comprehensive ACB study conducted by Buligan (1929) showed six larval instars while Camarao (1976) indicated five larval instars. The Laguna population was used in both studies. In another life history study using the Bukidnon population, results showed significant differences in larval and pupal durations when grown on fresh corn leaves (Caasi-Lit 2007). The ACB from Bukidnon population underwent six larval instars. This contradicted the results in this present study where the ACB population from Bukidnon grown under artificial diet underwent only five larval instars.

As reported in previous works, the first larval instar begins from egg hatching to the first molts, as evidenced by the first head capsule shed off by the neonate larva (Esperk 2007). The second larval instar starts after the first molt to the second molt. The head capsule from the second molting is usually seen attached to the artificial medium inside the cup. Molting goes on until the last instar full-grown larva turns into a pupa.

Further study should be conducted on this unusual phenomenon of variable number of larval instars among local ACB populations.

Pupal period duration and length of pupa

Pupal period duration of the six ACB populations showed significant differences (Table 2). ACB from Bukidnon had the longest pupal period (8.34 days) followed by ACB from Isabela (7.27 days). The rest of the ACB from Laguna, Camarines Sur, Leyte and South Cotabato had statistically similar pupal period durations.

The pupal lengths (Table 5) significantly differed among locations with the populations of ACB from Laguna as the longest (13.84 mm) followed by those from Bukidnon (13.61). The shortest pupae were from South Cotabato and Leyte. Differences in the duration of pupal period and pupal length are important because

Table 5. Pupal length (mm) of the different populations of the Asian corn borer, *Ostrinia furnacalis* (Guenee), from six regional sites in the Philippines.

Collection Sites (Provinces)	Pupal Length (mm)
Isabela	13.26 ± 0.08^{bc}
Laguna	13.84 ± 0.19^{a}
Camarines Sur	13.55 ± 0.09^{ab}
Leyte	13.25 ± 0.24^{bc}
South Cotabato	13.25 ± 0.11^{c}
Bukidnon	13.61 ± 0.13^{a}

Values (mean $\pm$ SD) in the same column, having same letters are not significantly different at $\alpha = 0.05$ LSD.

they may indicate adult characteristics affecting future population viability most likely pupal length or size indirectly related to the size of emerging adult which, in turn, may be related to longevity, female fecundity or other reproductive aspects. On the other hand, pupal period can affect synchrony of male and female emergence in addition to development or maturation of reproductive organs. i.e. ovaries and testes, as well as egg and sperm quality.

Overall, results showed variations in the total developmental period from egg hatching to adult emergence in decreasing order: Leyte > Laguna > Bukidnon > Camarines Sur > South Cotabato > Isabela and for pupal length, Laguna > Bukidnon > Camarines Sur > Isabela > Leyte > South Cotabato.

Differences in the length of larval period and number of larval instars may be attributed to factors such as maternal age, quality of the egg, the kind of host or diet as reported by Esperk (2007). There may be other local populations of *Ostrinia* in the country. These significant variations observed in the total larval period and number of larval instars are interesting researchable aspects to pursue in the future.

The possibility that ecotypes exist in different places may explain the variations observed in the present study which are in agreement with the findings of Demayo (1998), Barrion et al. (1981) and Mendoza et al. (1994). Cytogenetic analysis of local ACB populations from Leyte, Bukidnon and South Cotabato revealed significant differences in meiotic indices, haploid numbers and other parameters (Barrion et al 2011 In press). This was further confirmed in the genetic variations observed within and among the three ACB populations. Based on these parameters, there are indeed variations existing among local populations of the ACB. A research work to study this variation at the molecular level is in progress.

In evolutionary biology, the evolution of adaptations is a product that is based on variations serving as the raw material upon which natural selection acts. Selection factors can include natural enemies as well as host plant qualities. In pest management, this implies that any control strategy should consider the existence and extent of variation within a pest population. The latter would be most significant when the introduction of a new pest management technology would be exposed to factors conducive to efficacy breakdown or possible development of pest resistance. This scenario is exemplified in the work of Demayo (1998) where he showed that the variation in host utilization and response to various Bt-endotoxins among rice stemborers was population-specific. These variations were indicative of genetic structure of rice stemborer populations. He further suggested that the population-based variations indicated that Bt-rice will not have the assurance of complete success when deployed in different localities.

Further implication of the results of the present study is the possible breakdown of resistance of Bt corn against the ACB in the Isabela population which had the shortest life cycle. The breakdown of host resistance (or development of insect resistance) would be faster considering the regular practice of farmers of planting corn in three cropping seasons per year. Rates of evolution of adaptations vary within species and populations. However, it is known that the shorter life and generation cycles coupled with increased selection pressures due to increased mortality favor faster rates of evolutionary changes, which may include development of insect resistance.

Considering such findings, a comprehensive assessment of variations among ACB populations would greatly benefit the durability of the Bt corn technology by providing baseline information on local ACB populations that maybe useful in formulating locality-specific insect resistance management strategies.

SUMMARY AND CONCLUSION

The life cycle and larval instar variations of the most important insect pest of corn, *Ostrinia furnacalis* (Guenee), in six local populations were studied. F2 generation of laboratory reared test insects were used and they were reared under normal room temperature and relative humidity. Variations among the different local populations of the ACB were observed for the length of life cycle, number of larval instars, pupal duration and pupal length. The ACB populations from Laguna and Leyte had the longest life cycle while that from Isabela had the shortest. There were only five larval instars for the populations from Isabela, Camarines Sur, South Cotabato and Bukidnon while six larval instars were recorded for those from Laguna and Leyte. Pupal duration (days) and length (mm) significantly differed among the six populations.

The variations observed in the length of life cycle and number of larval instars among the six local ACB populations studied maybe indicating existence of ecotypes in different corn-growing areas in the country. Variation in the local populations is possible as they are affected by the differences in environmental conditions in the various geographical regions. This is most significant when new host plant genotypes or crop varieties are introduced as in the case of Bt corn which had been commercially planted for the last ten years. It is therefore recommended that further studies be done to expand or build on the data already available which suggest the existence of ecotypic variations among local populations of ACB in the Philippines.

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