Nigerian Journal of Ecology (2014) 13:12-18.

ISSN: 1116-753X

General performance and cocoon yields of two hybrids of *Bombyx mori* L. (Lepidoptera: Bombicidae) fed on leaves from pruned and unpruned mulberry plants

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(Accepted 10 March 2014)

ABSTRACT
Cocoon production by two silkworm, Bombyx mori L. (Lepidoptera: Bombicidae) hybrids fed on leaves from pruned and unpruned mulberry plants was investigated in a 2 x 2 factorial experiment fitted into a completely randomized design with four replicates. The aim of the experiment was to investigate whether mulberry leaf pruning has any effect on cocoon production optimization. Each replicate consisted of one hundred silkworm larvae of each hybrid which were fed throughout the larval stages on leaves collected from pruned and unpruned mulberry plants. Results showed that pruning of mulberry plants and silkworm hybrids influenced the number of cocoons produced. Larval and pupal weights, number of cocoons, single cocoon weight and shell weight were significantly higher when the silkworms were fed on leaves from pruned plants, while percentage mortality was significantly lower. However, fecundity and larval emergence were not influenced by any of the two factors or in combination.

Key words: silkworm, cocoon yield, pruning, mulberry plant, leaves

INTRODUCTION
Mulberry is a fast growing plant and its leaves can be harvested several times in a year. It is a perennial plant and can remain in a plot for 15 to 20 years (Ullal and Narasimhanna, 1987). For a perennial plant like mulberry, pruning is one of the agronomic practices that ensure regular production of succulent and quality leaves (Jolly, 1986). Pruning induces luxuriant growth in the plant; it can also be used to adjust leaf production period to synchronize with silkworm rearing or to extend the leaf production period (Krishnaswami *et al*., 1973). Pruning of mulberry plant significantly improves chlorophyll, total soluble protein, RUBPKs, RNA, and starch contents of mulberry leaves (Yamashita and Fujino, 1986).

Since silkworm takes from mulberry leaves necessary nutrients for its growth and development and for silk production (Machii and Katagiri, 1991; Bahar *et al*., 2011), the quality of mulberry leaf is very crucial in the production of quality cocoons. In fact, poor leaf selection results in malnutrition, poor growth, and poor silk and cocoon production (Krishnaswami, 1979). Leaf pruning provides succulent leaves needed to feed young worms. The early larval instars are usually fed on tender succulent leaves which contain sugar, fewer amounts of fiber, starch, and high moisture and protein contents, while later instars are fed on mature leaves (Bahar *et al.*, 2011). In this study, the impact of mulberry leaf pruning on silkworm cocoon production was investigated.

MATERIALS AND METHODS

Experimental set-up

The study was carried out in the Sericulture Laboratory, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The eggs of the five silkworm species used were obtained from Ekiti State Sericulture Centre Ado-Ekiti, Nigeria, while the mulberry plant variety S30 accessioned in FRIN was used for the feeding of the silkworm species. The experiment consisted of two factors: (a) two hybrids of silkworm – W_1D_2 and $C_1J_{2;}$ and (b) two levels of pruning - pruned and unpruned in a 2 x 2 factorial arrangement fitted into a completely randomized design with four replicates. The mulberry leaves were harvested between 8 and 10 am and preserved in transparent polythene bags for the different preservation periods at ordinary room temperature and humidity (28±2^{o}C; 70%RH) for 19-21 days. Leaf samples from each treatment were randomly taken from the preserved mulberry leaves. The samples were weighed, oven-dried to constant weight and allowed to cool. They were thereafter ground to fine powder and analyzed for nutrient contents as described by the Association of Official Analytical Chemist (AOAC, 1998).

Silkworm is known to react negatively to environmental conditions, especially temperature and relative humidity. These factors were closely monitored during the cause of these experiments and maintained at 23-26^{o}C and 73-86% RH using the standard suggested by Ullal and Narasimhanna (1987) and Suresh *et al*. (2007).

Preparation of eggs for hatching

Diapausing eggs of the five silkworm hybrids were preserved in refrigerator at 2.5-5^{o}C. The eggs were removed from the refrigerator as need arose. Prior to the removal of the eggs for rearing, the temperature of the refrigerator was raised from 5 to 15^{o}C for 3 hours to avoid shock. Thereafter, the egg cards were rinsed in 2% formalin solution for 15 minutes and later washed in running water. The egg cards were then kept between two large sheets of filter paper to dry and subsequently immersed in glass trough of Hydrochloric acid (HCl) (1.10 specific gravity) at 29^{o}C for 50 minutes (cold acid treatment) to break the diapause (Jolly, 1988). Thereafter, the eggs were removed from the acid and rinsed under running water for 5 minutes until the acid was completely washed off. For incubation, the egg cards were then turned upside down on large paper spread in a rearing tray on which old newspaper sheets were spread.

Twenty four hours later, previously disinfected foam pads were soaked in water and arranged at the four sides of the rearing tray before covering with paraffin or nylon paper. This was to maintain high humidity and temperature for 10-11 days, while the colour change of the eggs was observed. When eggs were freshly laid, they were yellow in colour but turned brown 24 hours later ushering in hibernation and it was at this stage they were acid-treated. When the egg colour turned blue or at the egg pigmentation day, the egg cards were kept in total darkness for 2 - 3 days, a process called 'black boxing'(Jolly, 1986). On the 11th day of incubation, the eggs were exposed to bright light early in the morning. This was done to achieve uniform hatching because darkness arrests hatching of the developed eggs and facilitates lagging embryo to reach hatching phase. The egg cards were spread uniformly in one layer on paraffin paper.

Brushing of newly hatched eggs.

After two hours of exposing the eggs to light in the morning, chopped leaves (0.5 cm x 0.5 cm) were sprinkled in a single layer over the hatched larvae for about 10-20 minutes to allow the larvae to crawl on the cut leaves. The egg sheets were turned upside down and held about 5 cm above the rearing tray with paraffin paper and taped gently to complete the process of transferring the larvae (brushing).

Preparing rearing house and equipment

Five days to the commencement of brushing, the rearing room, rearing trays, and all other equipment were washed, dried and disinfected with 3% formalin solution. After brushing, one hundred silkworm larvae were randomly selected from each of the five hybrids and fed four times at 8.00 hrs, 11.00 hrs, 14.00 hrs, and 17.00 hrs on leaves from pruned or unpruned mulberry plants until they passed the five larval stages. Treatment trays were randomly distributed on the rearing racks, and thereafter, larval mortality was determined.

Cleaning and caring for the worms

To maintain hygiene in the rearing bed, dirt such as left-over or rejected leaves, fecal matters and carcasses of the third, fourth and fifth instar larvae were removed from the rearing trays on a daily basis, and covered with clean nets. Mulberry leaves were broadcast on the net after the cleaning procedures, making the larvae to climb onto the net from the trays and hang onto them while trying to reach and feed on the fresh leaves. The larvae on the net were then transferred into other clean trays or cleaned old trays, and thereafter, clean sheets of paper were spread on them. This procedure was not carried out on the first and second instars which were fragile so as not to lose them.

At the commencement of each moulting, the larvae were dusted with slaked lime ($CaCO_3$) to dry up the bed and leaves so that all the larvae could stop feeding. Dusting also reduced the chances of the growth of mould and other disease-causing micro-organisms

Mounting of mature worms and cocoon processing

A day before the commencement of cocoon formation, fully grown silkworm larvae were later mounted on montages for cocoon spinning. All cocoons were harvested at six days old, out of which 20 were randomly selected from each replicate for the determination of pupal weight, shell weight and shell ratio. The pupae were subsequently kept until the adults emerged. Percentage adult emergence was calculated using the formula:

$$\frac{\text{Number of emerged adults}}{\text{total number of pupae.}} \times 100$$

Freshly emerged male and female adults were placed on rearing trays for mating for three hours according to the different treatments in four replications. The females were later separated and put in cellule on thick brown cardboard paper where they laid their eggs. After 24 hours, newly laid eggs were acid–treated and incubated, and the newly hatched larvae were brushed (Ullal and Narasimhanna, 1987)

The number of hatched eggs was determined from the number of egg shells recovered. After hatching, the egg shells became white, un-hatched eggs became black, while unfertilized eggs retained their yellow colour. The number of hatched eggs was therefore calculated as percentage of the total eggs laid. Other data taken were: larval mortality, larval developmental period, larval weight, pupal weight, percentage larval emergence, and fecundity, In order to determine longevity of adult silkworm, five pairs (males and females) were kept separately in Kilner jars till death, and number of days from adult emergence to death represented longevity. The following formulae were used to calculate the data taken:

Larval developmental period = No of days from hatching to cocooning;

Larval weight = average weight of 10 larvae;

Pupal weight = average weight of 10 pupae;

Percentage larval emergence

$$= \frac{\text{No of emerged larvae}}{\text{Total number of eggs}} \times 100,$$

Fecundity = Total no of eggs laid by one adult female.

All data collected were subjected to analysis of variance (ANOVA) test at $P < 0.05$. Least Significant Difference (LSD) was used to compare the means.

RESULTS

Pruning of mulberry plants and silkworm hybrid jointly influenced the number of cocoons produced (Table 1). Both silkworm hybrids produced heavier cocoons when fed with pruned leaves than when fed with unpruned leaves. Number of cocoons produced by W_1D_2 was lower irrespective of pruning status of the mulberry plants; the hybrid produced more cocoons when fed with pruned leaves than otherwise (Table 1).

Cocoon shell weight was higher when fed with pruned leaves than when fed with the unpruned. The shell ratio was however, not influenced either jointly or singly by these two factors (Table 2). Both silkworm species produced higher larval and pupal weights when fed with leaves from pruned plants. Fecundity and larval emergence were not influenced by any or both of the two factors (Table 2). Mortality was significantly lower when silkworm W_1D_2 was fed on leaves from pruned plants. However, pruning did not influence mortality in silkworm hybrid C_1J_2 (Table 2).

DISCUSSION

Pruning is one of the most important agronomic practices of mulberry cultivation. When plants are pruned, more nutrients are translocated to new sites to produce new branches and more succulent leaves. Pruning of mulberry plants ensures the production of fresh and succulent leaves (Ullal and Narasimhanna, 1987; Wang, 2006). Results from this experiment showed that single cocoon weight, shell weight, larval and pupal weight were significantly higher when silkworms were fed with pruned mulberry leaves than the unpruned. Yamashita and Fujino (1986); Wang (2006, 2009) recorded increase in chlorophyll, CO_2 fixation, total soluble protein RUBPcase,

Table 1: Cocoon yields of two silkworm hybrids fed with leaves from pruned and unpruned mulberry plants

Variable	Silkworm hybrid	Mulberry leaves		
		Pruned	Unpruned	Mean
Number of Cocoons	C_1J_2	95.7	95.5	86.0
	W_1D_2	78.2	62.5	70.4
	Mean	86.9	79.2	
		$LSD_{(0.05)}$ S=7.5*; L=7.5*; SxL=10.7*		
Single cocoon weight (g)	C_1J_2	1.29	1.02	1.15
	W_1D_2	1.22	1.07	1.14
	Mean	1.25	1.04	
		$LSD_{(0.05)}$ S = 0.08; L = 0.08*; SxL = 0.11		
Shell weight (g)	C_1J_2	0.26	0.17	0.21
	W_1D_2	0.21	0.18	0.20
	Mean	0.23	0.18	
		$LSD_{(0.05)}$ S = 0.03; L = 0.03*; SxL=0.05		
Shell ratio	C_1J_2	19.85	18.45	19.15
	W_1D_2	18.25	18.50	18.37
	Mean	19.05	18.47	
		$LSD_{(0.05)}$ S = 2.4; L = 2.4; SxL = 3.4		

Values are means of four replicates. LSD's are for the following comparisons. S, Silkworm hybrids; L, Mulberry leaves; SxL= Interaction between Silkworm hybrids and Mulberry leaves; * significant at P < 0.05.

total nitrogen, starch and RNA-P when mulberry plants were pruned. This implies that pruned leaves are richer in water, minerals and carbohydraetes than the unpruned. The authors also observed increase by more than 10 percent in leaf weight compared with unpruned plants.

Soft and succulent tender leaves had been noted to be a good source of water and protein (Bahar, 2011) which are essential in cocoon production. Tenderness of mulberry leaves is also important because it makes the leaves attractive to the silkworm for feeding (Tribhuwan and Mathur, 1989). Similarly, results showed that mortality was significantly lower, while larval and pupal weights were higher, when silkworms were fed on leaves from pruned mulberry trees compared to the unpruned ones. This lends credence to the fact that the quality of leaves from pruned mulberry plant is higher than that from the unprunned ones, and high leaf quality is a prerequisite for healthy silkworm growth and high quality cocoons (Venkalaramu, 1987; Kherdeker *et al.*, 2000). It is therefore recommended that pruning of mulberry plants should be encouraged to keep the plants in check and in order obtain high silkworm cocoon yield.

Table 2: Develomental characteristics of two silkworm hybrids fed with leaves from pruned or unpruned mulberry plant

Variables	Silkworm hybrid	Mulberry leaves		
		Pruned	Unpruned	Mean
Larval weight (g)	C_1J_2	3.01	2.86	2.94
	W_1D_2	3.06	2.91	2.99
	Mean	3.04	2.89	
		$LSD_{(0.05)}$ S = 0.14; L = 0.14*; SxL = 0.2		
Pupal weight (g)	C_1J_2	1.03	0.86	0.94
	W_1D_2	0.95	0.96	0.95
	Mean	0.99	0.91	
		$LSD_{(0.05)}$ S = 0.08; L = 0.08*; SxL = 0.11*		
Fecundity	C_1J_2	443	411	427
	W_1D_2	438	382	410
	Mean	440	397	
		$LSD_{(0.05)}$ S = 75.7; L = 75.7; SxL = 107.0		
Larval emergence (%)	C_1J_2	98.37	98.55	98.46
	W_1D_2	98.50	99.05	98.78
	Mean	98.44	98.80	
		$LSD_{(0.05)}$ S = 0.89; L = 0.89; SxL = 1.25		
Adult emergence (%)	C_1J_2	91.2	71.2	81.2
	W_1D_2	72.5	78.7	75.6
	Mean	81.9	75.0	
		$LSD_{(0.05)}$ S = 12.5; L = 12.5; SxL = 17.7*		
Mortality (%)	C_1J_2	4.5	4.0	4.2
	W_1D_2	21.8	37.5	29.6
	Mean	13.1	20.8	
		$LSD_{(0.05)}$ S = 7.6*; L = 7.6*; SxL = 10.7*		

Values are means of four replicates. LSD's are for the following comparisons. S, Silkworm hybrids; L, Mulberry leaves; SxL= Interaction between silkworm hybrids and mulberry leaves; *, significant at $P < 0.05$.

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