Biology of Cyllodes bifacies Walker (Coleoptera: Cucujoidea: Nitidulidae), a Pest of Oyster Mushroom (Pleurotus ostreatus) in Sri Lanka

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ABSTRACT. <u>Pleurotus ostreatus</u> cultivation in Kandy district is severely affected by larvae of <u>Cyllodes bifacies</u> (Walker). The larval instars as well as adults, both male and female, feed on mushroom fruiting bodies. Biology of this species was studied under laboratory conditions $(25\pm2^{\circ}C \text{ and } 80\pm2\% \text{ RH})$ at Peradeniya.

Adult was 2.4 - 3.7 mm long oval convex, female laid oval shaped, white eggs about 0.94 mm long and 0.29 mm wide in clusters of 4 or 8 eggs, inside the tissue of fruiting bodies. Mean hatchability and incubation period of eggs were 92% and 21 ± 2 hours, respectively. Larvae possessed distinct head bearing chewing mouthparts, three thoracic legs, paired urogomphii and transverse rows of curved spines in anal segment.

There were three larval instars. The mean larval, prepupal and pupal period were observed as 3.28 ± 0.2 , 5.7 ± 0.81 , 4.4 ± 1.3 days respectively at $25\pm2^{\circ}$ C. Adults always emerged in the morning and started to oviposit just after copulation, which occurred in 8 - 9 days after emergence.

Fecundity was 95.6 for first 40 days. Longevity was 122 days for male and 128 days for female under laboratory conditions.

A greater damage is caused by the larval feeding. When larval attack occurred at the pinhead stages, there were no visible symptoms on the developing fruiting bodies. All affected fruiting bodies were collapsed, turnea yellow and finally perished 3-5 days after the attack.

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INTRODUCTION

One of the major limiting factors in the cultivation of mushrooms is the various insect pests (Locatelli, et al., 1994), such as Phorid fly (Mohan et al., 1995; Clift, 1979; Disney, 1993; Johal and Disney, 1994), Sciarid fly (Clift, 1979; White, 1993; Sasakawa, 1992), Ceiced fly (Johal et al., 1991; Clift, 1979), Ciid beetle (Lawrence, 1991), Nitidulid beetle (Johal et al., 1992; Hayashi, 1978) and Tenebrioid beetle (Johal et al., 1993) that have been recorded in various places except in Sri Lanka.

A survey conducted in July 1993, to investigate the pests affecting *Pleurotus ostreatus*, revealed that nitidulid beetle *Cyllodes bifacies* (Walker) was one of the major pests that threatened this industry. The pest was reported to be prevalent throughout the year.

It is reported that Cyllodes biplagiatus (Boving and Roger) and C. alei, C. literatus infest Pleurotus sp. in Japan and C. whiteii infest Pleurotus Sajar caju in India (Johal et al., 1992), but nothing has been reported about C. bifacies.

This paper reports the results of laboratory studies on morphometrics, life cycle, longevity and fecundity of *C. bifacies*. These information will be useful in designing an appropriate management system for the pest.

MATERIALS AND METHODS

The larvae of *C. bifacies* were obtained from infested fruiting bodies of oyster mushrooms collected from a commercial farm in Kandy district, Sri Lanka, in July 1993. They were then mass cultured in the Entomology laboratory of the Department of Agricultural Biology, University of Peradeniya.

Oyster mushroom grown in polypropylene bags were kept in meshed, wooden cages measuring $75 \times 60 \times 60$ cm for the rearing of insects. The entire experiment was carried out at room temperature ($25\pm2^{\circ}$ C). Humidity in the cages were maintained at $80\pm2\%$ by providing wet bands. Beetle was identified up to the genus level using available published keys based on the adult and larval morphology.

Samples of the beetle were sent to the Natural History Museum, UK for species identification.

Randomly selected 605 adults were dissected out to expose external genitalia and the sex ratio of the species was calculated by examining the type of genitalia. Eggs were obtained by placing 40 newly emerged adults of *C. bifacies* adults in a glass jar for 24 hours with fresh mushroom fruiting bodies. Then the mushroom pieces were removed and dissected under a binocular microscope (4×12) in order to locate the eggs which were then measured with the help of an ocular micrometer.

The incubation period and hatchability were studied using mushroom fruiting bodies containing eggs. These eggs were collected after keeping the beetles on mushroom pieces for 6 hours. Eggs were kept in plastic petri dishes covered with perforated lids. Twenty five petri dishes were used. Number of larvae emerged was recorded after 13 hours and there after at one hour intervals. Evaluation of number and duration of larval instars were made by continuous collection of larval instars from the mushroom pieces containing eggs twice a day. Head capsule width of all larvae were measured using an ocular micrometer. A histogram was drawn to show the frequency distribution of head capsule width of possible larval instars and the number of larval instars was determined from the peaks. These results were confirmed by using Dayer's law.

Plastic petri dishes were partially filled with substrate (compost medium used for growing mushrooms) and five prepupae of *Cyllodes bifacies* were placed in each petri dish and covered with perforated plastic lid. Daily observations were made till the adults came out and average pupal duration was calculated.

Fecundity studies were carried out using day old adults reared in glass beakers covered with muslin cloths. They were supplied with fresh pieces of mushroom fruiting bodies daily and the ones kept on previous day were dissected out and checked for the presence of eggs. Fecundity of the adult was determined by counting the number of eggs laid by each female. Preovipositional period was evaluated from the day when the eggs were noticed first, using the same procedure. Larval and pupal stages were prepared in 70% Ethyl alcohol and the larval instars were stained with 1% carbol fusion in order to observe chitinized morphological structures.

RESULTS AND DISCUSSION

Morphological features of Cyllodes bifacies

Adult beetle (Plate 1) is small, oval convex shape with length ranging from 2.5 - 3.7 mm. It has a hard, smooth elytra which is shiny black in colour with two dull yellow spots. Hind wing is membranous (Figure 1 c). Pronotum is the same dull yellow in colour with two black stripes in middle. Eleven segmented capitate antennae terminate with three clubbed segments (Plate 2). Tarsi five segmented; 5th segment is elongated; 1st, 2nd and 3rd segments are bilobed, 4th is reduced, hidden inside the 3rd segment (Figure 2, Plate 3). The elytra is shorter than the abdomen leaving the tip of the abdomen exposed.

Larva (3rd instar)

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Elongated, slightly flattened body with pigmented (slightly scleretized) head, protergum and 9th abdominal tergite; Lateral projections in abdominal segments; Nine pairs of annular biporus type spiracles placed at the end of spiracular tubes (Figure 2G). Ninth abdominal segment without spiracle or pregomphii but with paired upwardly directed urogomphii bearing four small setae. Biforked anal segment bearing transverse raws of fine curved spines (Plate 5, Figure 2G and F).

Head transverse, 0.87 mm in width, widen at base; four ocelli in two raws; 3 segmented antennae which is about 0.184 mm in length (1st seg. 0.048 mm, 2nd seg. 0.076 mm, 3rd seg. 0.06 mm) (Plate 7). Mouth parts with small labrum, well developed mandible, strongly twisted apically; Prostheca and mola aspirated with teeth: maxilla with 4 segmented palpi: labium with one segmented palp (Figure 2A and B).

Three pairs of five segmented thoracic walking legs which are about 4.687 mm in length (Coxa:1 mm, trochanter:0.571 mm, femur:0.6 mm, tibia:0.428 mm, tarsungulus:0.088 mm) (Figure 2E). Slender tarsungulas moderately curved, with simple asymmetrical two setae (Plate 6).

According to the morphological characterization, beetle was identified as Cyllodes bifacies (Walker).

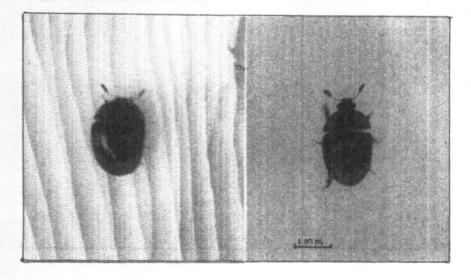


Plate 1.

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Cyllodes bifacies Walker.

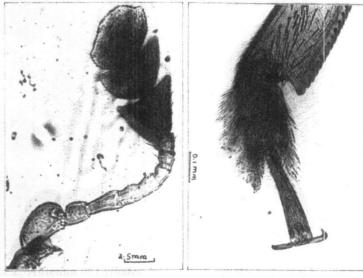
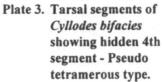
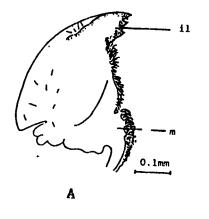
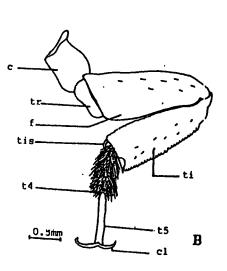
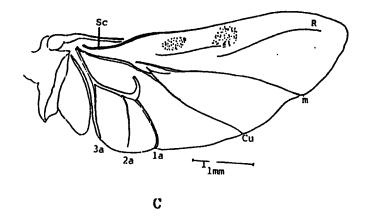


Plate 2. Antenna of Cyllodes bifacies.











(A) Left mandible; (B) Proleg; (C) Hind wing. c- coxae; cl- claw; f- femur; il- incisor lobe; m- molar region; ti- tibia; tis- tibial spur; trtrochanter; $t_4 - 4^{th}$ tarsal segment; $t_5 - 5^{th}$ tarsal segment.

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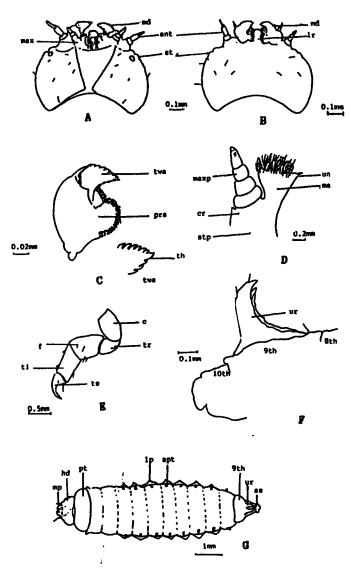


Figure 2.

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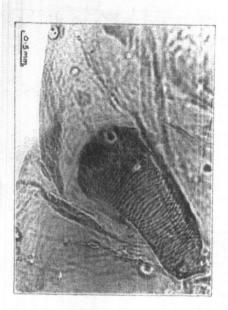
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Cyllodes bifacies - Third instar larva.

A) Head capsule-ventral view; B) Head capsule-dorsal view; C) Left mandible; D) Left maxillae; E) Mesothoracic leg; F) Posterior abdominal segments showing Urogomphii; G) Larva - habitus.

ant- antenna; as- anal segment; cr- cardo; Hd- head; Ip- lateral projection; ir- labrum; mamala(united lacinia and galea); maxp- maxillae; md- mandible; mp- mouth parts; prsprostheca; pt-protergum; spt- spiracular tube; st- stemmata; stp- stipe; ts- tarsangulus; thteeth; twa- twisted apical part; t9- 9th turgite; un- uncus; ur- urogomphus; 8th- 8th abdominal segment; 9th- 9th abdominal segment and 10th- 10th abdominal segment.

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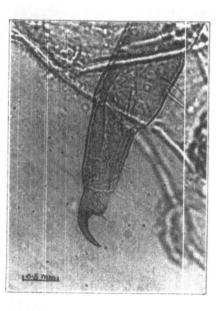


Plate 4. Spiracle of Cyllodes bifacies larva.

Plate 6. Tarsungulus of larva.

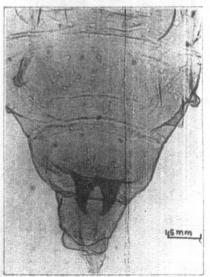


Plate 5. Post-abdominal segments of larva showing urogomphii and fine spines in anal segment.

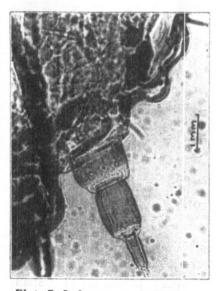


Plate 7. Left antenna of of larva

The key for identification of Cyllodes spp. attacking Pleurotus spp. (modified from Jhoal et al., 1992).

1. Ninth abdominal tergum with a small protuberance or pregomphii medially; dorsal sclerite absent. Tarsungulus of each leg moderately produced basally. *C. ater.*(Herbst)

Ninth abdominal tergum without pregomphi medially, dorsal sclerite present, Tarsungulus of each leg well developed basally 2

2. Abdominal spiracular tubes short urogomphii moderately developed. Tarsungulus strikingly produced basally.

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Abdominal spiracular tube long urogomphii very well developed 3

Urogomphii with very short setae on the outer margin in the anterior part. Tarsungulus of each leg is slender. C. bifacies (Walker)

4. Mandibular apex twisted nearly 90°, prostheca reduced to small pad with indistinct series of minute denticle. *C. biplagiatus* (le Conte)

Mandibular apex strongly twisted apically, prostheca and mola strongly aspirated. C. literatus (Reitter)

External appearance of the male and female was similar except for the external genitalia. Sex ratio of the species was 1:1 (Table 1).

Table 1.	Estimation of sex ratio and percentage of females in the
	population of Cyllodes bifacies.

Total No. of adults observed	No. of Male	No. of Female	Ratio	. % of Female
605	318	287	1:0:97	47%

This 1:1 ratio was proved by using Chi square test (P = 0.05).

Observations on the life cycle of Cyllodes bifacles

Eggs

Eggs were oval shaped, white in colour and about 0.94 mm long and 0.29 mm wide. They were deposited inside the tissue of the fruiting bodies in clusters of 4 - 8 eggs. Mean incubation period of eggs was 21 ± 2 hours at $24\pm 2^{\circ}$ C. The mean hatchability of eggs was 92%.

Larval stages

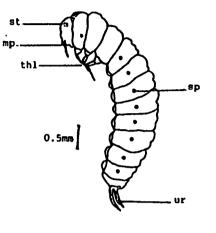
The mean larval period was 3.28 ± 0.4 days at $24\pm2^{\circ}$ C. They moult two times and pass through three larval instars (Figure 4). Newly hatched larvae is also microscopic. Mean head capsule breadth of first instar larva was 0.45 ± 0.025 mm. Just after hatching they start feeding on mushroom and moult within 20 hours. Mean head capsule breadth of 2^{nd} instar larva was 0.6260 ± 0.026 mm. Third larval instar was 8.8 mm long and 1.9 mm wide. Mean head capsule breadth was 0.854 ± 0.37 mm and 1.6 times as wide as long (Figure 2A and B).

Prepupal stage

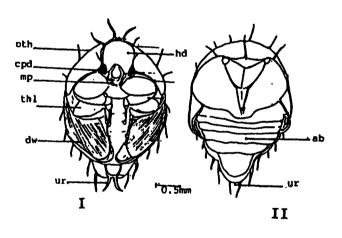
The mature larvae became active and stopped feeding, then moved into the compost mainly through stalk and sometimes with dropped fruiting bodies. Then it became inactive and its length was reduced and body became as 'C' shaped. (mean body length of prepupal stage was 4.5 mm) (Figure 3A). Mean duration of the prepupal stage was 5.7 ± 0.81 days at $25\pm2^{\circ}$ C.

Pupal stage

Pupae was soft exarate type. The colour of the pupae changed from white to pale yellow and developing wing pads became visible within 3 - 4 days after pupation (Figure 3B). When the pupae was fully matured the colour became brown and the wings turned black. This happened 5 - 6 days after pupation. Mean pupal period was 4.4 ± 1.3 days at $25\pm2^{\circ}$ C.



A



B

Figure 3. (A) Prepupal stage of C. *bifacies*, (B) Pupal stage of C. *bifacies* (I. Ventral view. II. Dorsal view).

cpd- compound eye; dw- developing wing; prth- prothorax; mp- mouth parts; sp- spiracle; thl- thoracic legs; ur- urogamphui.

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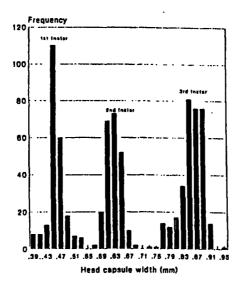


Figure 4. Frequency distribution of head capsule width of *Cyllodes* bifacies Larvae.

Adult

Adults always emerged in the early hours of the day (before 9.00 h). Newly emerged adults were pale reddish brown in colour, without any markings on the body. Fourth day from emergence they acquired their characteristic appearance. They fed on fruiting bodies vigorously during this period.

Under laboratory condition the life cycle of *C. bifacies* lasts about 15.3 ± 1.8 days. Copulation occurred 8 - 9 days after emergence. Then the female started to oviposit just after the copulation. Mean fecundity of adults reared on *Pleurotus ostreatus* for first 40 days after emergence was 95.6. Longevity of male was 122 days and female was 128 days in the laboratory.

Damage

Main damage caused to the mushrooms is feeding on fruiting bodies and hollowing out of the stipe. *C. bifacies* is mycophagous and both the larval and adult stages fed on mushroom fruiting bodies.

In the case of larva, both mandibles are strongly twisted apically. Therefore the cutting edges is turned to the outer margin of the apex (Plate 7), and inner margin of the mandible possess articulated lobe called prostheca which is aspirated with several teeth (Figure 2C). By means of these structure they chew the tissue of the fruiting body. Finally the fruiting body become detached 3-5 days after the infestation.

As there were no visible symptoms on the fruiting bodies up to 4 days after initiation of fruiting bodies, a complete loss of crop was observed when the larval density exceed beyond 20 per fruiting body of about 10 cm diameter. Sometimes infested mushrooms might be packed for sale without knowing about the pest attack, and the larvae will be visible within 3 days.

CONCLUSION

Cyllodes bifacies is as a key pest in mushroom culture in Sri Lanka. Oval shaped, white eggs about 0.94 mm long and 0.29 mm wide were laid in clusters of 4 or 8 eggs, inside the tissue of fruiting body. Mean viability and incubation period of hatching of eggs were 92% and 21 ± 2 hours respectively. The mean larval, prepupal and pupal period were found to be 3.28 ± 0.2 , 5.7 ± 0.81 , 4.4 ± 1.3 days respectively at 25 ± 2 . Fecundity was 95.6 for first 40 days and longevity was 122 days for male and 128 days for female at laboratory condition.

A greater damage is caused by the larval feeding. When larval attack occurred at the pinhead stages, even though there were no visible symptoms on the developing fruiting bodies, these collapsed and turned yellow in colour and finally perished 3 - 5 days after the attack. The whole life-cycle of the insect is spent on mushroom cultures. The females have high fecundity and long period of longevity. Eggs showed short incubation period and high percentage of hatchability. Post embryonic development period was short. All these features may have led this species to develop in to a key pest of mushrooms.

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