A comparative evaluation of locally available substrates for rearing and studying biology of sciarid fly, *Lycoriella mali*

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**ABSTRACT**

An evaluation of methods for rearing sciarid flies, *Lycoriella mali*, was conducted in the laboratory. Five different substrates namely cotton waste with oyster mycelia, cotton waste only, oyster mycelia growing on Potato Dextrose Agar (PDA), fresh mushroom fruit and rotting mushroom fruit were tested for ability to support growth and development of the sciarid fly. The percentage survival rate for the adult sciarid fly was in the order cotton waste substrate and cotton waste with oyster mycelia being most suitable, both recording about 95% survival rate. These were followed by rotting oyster mushroom fruit and fresh oyster mushroom fruit with a mean survival rate of 70%. Oyster mycelium growing on PDA was not supportive of the adult flies but the larval stages with mean survival percentages of 30 and 70 respectively. The opposite was true for fresh oyster mushroom fruit which recorded the least mean larval survival rate of 20%. The best economical substrate for rearing *L. mali* in the laboratory was concluded to be cotton waste which scored high survival rates for both larval and adult stages. Life cycle studies on *L. mali* revealed a total duration of 23 days from the egg to the adult fly stages with the larval instars taking about three quarters duration of the life cycle.

**Key words:** Fungi gnats, growth, oyster mushroom, cotton waste.

**INTRODUCTION**

Sciarid flies, *Lycoriella mali* (Fitch), are economically important pests in mushroom farming. They are considered the most damaging mushroom flies in Zimbabwe (Practical Action, 2007). Sciarid fly damage was reported to have reduced mushroom production by 70% (Beyer et al., 1999) and an estimated yield reduction of $USD544 million from 1987 – 1988 in Pennsylvania State (Mehelis, 1995). These fungus gnats can affect all stages in mushroom development from spawning to fruiting (Rimker et al., 1993). This is due to the presence of two different active life stages, the larvae and the adult with different behavioural and feeding habits.

The sciarid larvae are gregarious oyster mycelial feeders which lead to the reduction of crop development and yield (Kiel, 2002). They feed on the organic substrate and in the process render it less suitable for the growth of the mushroom mycelia (Binns, 1980). The sciarid larvae can tunnel into the mushroom stipe, leading to a condition called “black stem” (Kiel, 2002). This reduces the aesthetic value of the mushroom resulting in commercial loss. The larval excreta of the sciarid were shown to inhibit mycelial growth (Cantelo and San Antonio, 1982).

The adult sciarid flies are not active mushroom feeders, but usually take in some minute quantities of water and other liquids from the mushroom (Wuest and Bengston, 1982). The adult flies can spread mushroom pathogens leading to rots and growth retardation of the mushroom. Some of the pathogens transmitted include *Trichoderma, Verticillium fungicola, Pseudomonas tolaasii* and *Pythium* (Kiel, 2002). Pyemotid mites and nematodes may also be transported by the adult flies (Wuest and Bengston, 1982). The extensive damage caused by the sciarid fly necessitates the study of its biology, rearing and control. Several studies have been done on the rearing of fungus gnats including the use of fungi namely *Phoma betae, Fusarium* species, *Botrytis*...
cinerea, Agaricus, Pleurotus and organic materials such as coconut and wood fibres to support growth of the flies (O’Connor and Keil, 2005; Kuhne and Heller, 2009). The studies have been performed mainly in the Western countries with conditions suitable for button mushroom cultivation and using substrates not readily available in Zimbabwe. The tropical climatic conditions prevailing in Zimbabwe favour oyster mushroom production which is also prone to sciarid fly attack. To date, no work has been recorded in Zimbabwe on the rearing of sciarid flies. There are several locally available potential organic substrates for sciarid fly rearing, which scientists have little appreciation in their role as food for sciarid flies.

The growth of mushroom farming industry in Zimbabwe makes continual availability of the fungus gnats for research necessary. The results of the present work would provide an insect colony for the studies on biology of the pest, which might lead to the discovery of more economic and effective methods of controlling the sciarid flies. For this reason, a study was carried out to investigate the ideal locally available substrates for successful and continuous rearing of the sciarid flies, L. mali, in the laboratory.

MATERIALS AND METHODS

Sourcing of test insects and study material

The sciarid flies and fresh mushroom fruit were obtained from mushroom bags in the mushroom growing room located in the Biological Sciences Department, University of Zimbabwe. Cotton waste material and oyster mushroom spawn (Pleurotus sajor-caju) were provided courtesy of the Mushroom project, Biological Sciences Department.

Adult L. mali flies were captured using a mouth aspirator as they moved around mushroom bags and placed in 375 ml glass jam jars before transferring them to the insectary room. The flies were captured during early morning hours when flies’ activity was low due to low temperatures.

Test to determine ideal substrate for adult L. mali flies

L. mali flies rearing was done in plastic jam jars (diameter 6 cm; height 13 cm), in the insectary room in the Biological Sciences Department. Five different substrates were tested if they could nurture and support the growth and development of L. mali adults: a) oyster mushroom mycelia growing on Potato Dextrose Agar (PDA), b) fresh oyster mushroom fruit, c) rotting oyster mushroom fruit, d) cotton waste material colonised with oyster mushroom mycelia and lastly e) cotton waste material alone. Approximately fifty (50 g) of each of the substrates were placed separately in jam jars and 20 adult sciarid flies introduced into the substrates using a mouth aspirator. This method was adopted from Lewandowski et al. (2004) with minor modifications. Five replicates were used for each substrate. The infested substrates were left in an isolator (cage) with 75% relative humidity and 25°C temperature and maintained for 36 h. These conditions resemble those in a typical mushroom growing house. The number of the surviving flies in each substrate was recorded.

Test to determine ideal substrates for L. mali larvae

Twenty, less delicate and gregarious feeding third-instar and early fourth-instar L. mali larvae were collected from infested mushroom substrate in growing room, using a plastic dropper, and placed in different substrates as above. The larvae were first washed off gently from the medium using distilled water from a dropper, then sucked up by the dropper and placed into the different media. Approximately fifty (50 g) of each of the substrates were placed separately in jam jars. Five replicates were used for each substrate. The infested substrates were left in the insectary room, in an isolator (cage) at 25°C and 75% RH for 72 h. The number of the surviving larvae in each treatment was recorded. An Analysis Of Variance (ANOVA) model was used to ascertain the statistical significance in the differences of the mean number of adult and larval survivals.

Determination of developmental time of L. mali life cycle stages

The results of the preceding experiments showed that cotton waste and cotton waste with mycelia produced the highest number of surviving larvae and adults. Therefore, cotton waste alone was chosen as the substrate for rearing L. mali in this part of the project. Twenty newly emerged adult sciarid flies (10 males and 10 females) were isolated and placed in each of the five jam jars with 50 g cotton waste material. After every 24 h, the cotton waste material was examined for sciarid eggs using a stereomicroscope. Twenty eggs were washed off using distilled water and placed into each of the five jam jars with 50 g cotton waste material. The infested substrates were left in the 25°C insectary room with 75% RH. The time of development from the eggs to the adult flies was monitored by sampling and scouting the jam jars and noting the time taken for the emergency of the various developmental stages of L. mali.

RESULTS

The results on the Table 1 show a significant effect (P < 0.05) of different substrates on both larval and adult survival. The lowest chances of survival were witnessed in the PDA colonised with oyster mycelia (6 adult flies) with relative medium survival chances for adult flies seen for the fresh oyster mushroom fruit (13 flies) and rotting oyster...
A summar of results (mean ± SE), n = 20, for the survivals of L. mali adults and larvae in different substrates.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Mean ± SE larval</th>
<th>Mean ± SE adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh oyster fruit</td>
<td>3.8 ± 0.8c</td>
<td>13.0 ± 1.6b</td>
</tr>
<tr>
<td>Rotting oyster fruit</td>
<td>19.0 ± 1.0a</td>
<td>14.0 ± 1.6b</td>
</tr>
<tr>
<td>Cotton waste only</td>
<td>19.8 ± 0.4a</td>
<td>19.6 ± 0.5a</td>
</tr>
<tr>
<td>Cotton waste + mycelia</td>
<td>19.6 ± 0.5a</td>
<td>19.8 ± 0.4a</td>
</tr>
<tr>
<td>PDA + oyster mycelia</td>
<td>14.2 ± 0.8b</td>
<td>6.0 ±1.0c</td>
</tr>
<tr>
<td>P-value</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values followed by the same letter in a column are not significantly different at 95% confidence level (p< 0.05).

<table>
<thead>
<tr>
<th>Life cycle stage</th>
<th>Mean time in days ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg – 1st instar larva</td>
<td>4 ± 0.63</td>
</tr>
<tr>
<td>1st instar larva – 2nd instar larva</td>
<td>3 ± 1.10</td>
</tr>
<tr>
<td>2nd instar larva – 3rd instar larva</td>
<td>3 ± 0.63</td>
</tr>
<tr>
<td>3rd instar larva – 4th instar larva</td>
<td>4 ± 0.63</td>
</tr>
<tr>
<td>4th instar larva – pupa</td>
<td>5 ± 0.63</td>
</tr>
<tr>
<td>Pupa – adult</td>
<td>4 ± 0.52</td>
</tr>
<tr>
<td>Average insect life span</td>
<td>23 ± 4.14</td>
</tr>
</tbody>
</table>

The choice of substrate for sciarid rearing is governed by the water retention properties and fibrosity of the material. This helps to reduce desiccation hazard to the adult, egg and young larval stages and also provides support for pupation of mature larval stages. Tung and Snetsinger (1970), used sterile cheese cloth, rye grains and spawn residue while other studies tried cotton wool, filter paper, nylon stocking material, cosmetic pad and facial tissue. Cotton waste material with and without oyster mycelia were found to be the most ideal substrates for survival of both larval and adult stages of L. mali. Besides providing cover for the fly from its texture, the cotton waste is highly nutritious and has good water retention properties ideal for the sciarid fly. Silva et al. (2002) reported cotton waste as a rich substrate in protein, lipids, carbohydrates, ash and fibre. The nutritional content of cotton waste, though not yet explored, has shown to support sciarid fly growth and development (Personal observations).

The use of oyster mycelium in cotton and PDA was derived

**DISCUSSION**

The highest chances of survival were seen for the cotton based cultures with an average of 19 flies which is equivalent to 95% insect survival rate. Tests for the effect of different substrates on larval survival showed that the lowest chances of survival were witnessed for the fresh oyster mushroom fruit. PDA colonised with oyster mycelia had relatively medium survival chances for larvae while high chances were observed for rotted oyster mushroom fruit culture and both cotton based substrates with an average of 19 larval insects surviving.

From the results, the comparative studies on survival of the two stages of the fly in culture media used were significantly different (P < 0.05). It could be deduced that rotted oyster mushroom fruit culture and the cotton based cultures were favourable media for survival of both adult and larval stages. However, the adult stage had better survival chances than larvae in the fresh oyster mushroom fruit culture. It was vice versa for the PDA colonised with oyster mycelia in which the larvae had better survival chances than the adult flies. The results confirm the choice of cotton waste material alone for subsequent experiments to study developmental stages of L. mali.

**Length of developmental stages of L. mali**

The average time taken for each life cycle stage of L. mali is shown on the Table 2 below. L. mali took an average of 23 days to complete its life cycle at room temperature. Shorter period of time was observed in-between larval instars. The period between the 4th larval instar and the pupal stage was the longest. Each instar stage took an average of 3 days and the sciarid fly spent most of its life time in the larval stage.
from the idea that sciarid flies are primarily mycophagous and wild mushrooms are their natural food (Richardson and Grewal, 1991). This was experimentally supported by earlier studies when inoculum of *Agaricus bisporus* was introduced into sciarid fly rearing flasks (O’Connor and Keil, 2005). The overgrown mycelium layer was shown to be hydrophobic and provides suitable substrate for oviposition (Tung and Snetsinger, 1970; Cantelo and San Antonio, 1982). This property of the mycelia also allows some water and fluids to settle on it which is then consumed by the adult flies. Kuhne and Heller (2009), reported that fungi with high mycelium growth are good food source for fungus gnats. They also showed that massive occurrence of fungus gnats in greenhouses was always associated with previous incidence of severe fungal mycelia growth in the growing media.

Sciarid larvae have voracious appetites and are more polyphagous than the adults (Meheisli, 1995). In this study, they thrived well in most of the tested substrates except in fresh oyster mushroom fruit. It was also observed that the larvae could dig into the PDA media and feed on it. This enabled them to survive even after grazing all the mycelia on PDA. However, in PDA with oyster mycelia, the sciarid larvae survival decreased as the mycelia overgrew. This observation agrees with studies by Cantelo and San Antonio (1982), who have shown *L. mali* larvae inhibition with growth of fungal mycelia.

Rotting mushroom was observed to support both *L. mali* fly stages better than fresh mushroom fruit. Freeman (1983), noted that females of sciarid flies are attracted to rotting vegetative matter, algae and fungi where they lay their eggs which hatch and emerging larvae continue to feed on decaying organic matter. Other findings recorded that sciarid larvae primarily survive on rotting plant matter (Richardson and Grewal, 1991) although they can cause damage to plant roots mostly of young plants and seedlings (Binns, 1980; Chandler et al., 2011). These previous studies support that larval *L. mali* prefers rotting to fresh organic matter. Fresh mushroom fruit on the other hand, has tough fibres which the larval stages find difficult to feed on and easily lose moisture and become dry with time.

The average time to complete *L. mali* life cycle was found to be 23 days, at 25°C. Musieba et al. (2012) stated that sciarid flies may complete life cycle in 25 days at 21°C and may take between 35 to 38 days at 18°C (Wetzel et al., 1982). This difference reflects that the speed of development of *L. mali* is temperature dependent. Wilkinson and Daugherty (year) found out that the optimum temperature for complete development of *Bradyisia impatiens* (a relative of *L. mali*) ranged from 18.9 – 30.0°C with mean development period of 19.2 days which increased with increasing temperature. Fungus gnats generally do not develop below 10°C while the highest temperature of 32.2°C was found to cause injurious effects on all stages of development (Harris et al., 1996). Kiel and Bartlett (1996), noted that the type of growing substrate used played a major role in speeding up development. In this study, the cotton waste substrate provided the sciarid flies with the essential nutrients to fasten development of the life cycle stages.

The longest stage in *L. mali* life cycle was between the 4th larval instar and the pupa, taking an average of 5 days. This stage takes more time to enable the sciarid larvae to obtain enough nutrients to develop fully into an adult and to survive throughout the pupal stage (Lewandowski et al., 2004). Shorter time was observed in-between the larval instars. This was mainly because these stages are separated by moults and are all active feeders, with no resting stage in-between them (McDonald, 1972).

Conclusions

The study has shown that cotton based substrates were the most ideal media for rearing *L. mali* flies. This waste is abundant at cotton ginneries throughout the country and was also shown to support mushroom growth. Therefore, mushroom growers using cotton waste as substrate for oyster production have to be prepared to eliminate sciarid pest problems. Further studies should focus on determination of nutritional requirements of *L. mali* which may be found in cotton seed hulls. Both growth stages of the fly were shown to prefer rotting to fresh mushroom fruit and be able to complete life cycle in 23 days at 25°C.

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REFERENCES


