Fungus gnat a common contaminant of insect culture in glasshouse and protected cultivation.

ABSTRACT

This study focuses on the identification and management of closely related fungus gnat species, *Bradysiaocellaris*C. and *Bradysia impatiens*J., within glasshouse insect cultures. Utilizing morphological and molecular techniques, we distinguish between these species, highlighting nuanced differences. The emergence of these gnats from plant soil poses challenges as they compete for food resources with target insects, acting as potential vectors for pathogens. To address these issues, comprehensive management practices, including hygiene maintenance, physical controls, targeted insecticides, and quarantine measures, are implemented. The study provides insights into effective pest control strategies for maintaining optimal conditions in controlled environment and ensuring the integrity of insect cultures.

Key words: Fungus gnats, *Bradysiaocellaris*, *Bradysia impatiens*, glasshouse, insect cultures, molecular, mtCOI, management.

Introduction:

Maintaining insect cultures in a glasshouse or protected cultivation environment necessitates the establishment of a controlled and conducive setting for the insects' growth and reproduction. Careful selection of insect species is paramount, considering factors such as temperature, humidity, and light requirements (Schowalter, 2022). Appropriate housing in well-ventilated containers or cages is crucial to prevent escapes and ensure the insects' containment. Providing a suitable substrate for egg-laying and larval development, along with a balanced and nutritious diet, is essential for the overall health of the insect culture (Cadinu et al., 2020). Environmental control measures, including maintaining optimal temperature and humidity levels through the use of heaters, fans, and misting systems, contribute significantly to successful insect cultivation (Cadinu et al., 2020). Biosecurity measures, such as quarantine and regular inspections, help prevent the introduction of pests or diseases that could compromise the insect culture. Rigorous monitoring, record-keeping, and maintaining cleanliness are key aspects of successful insect culture management. Attention to breeding conditions and reproductive cycles is vital for sustained populations. Research and experimentation within a controlled environment, following safety protocols, contribute to a deeper understanding of insect behavior and development. Waste management plans for the proper disposal of dead insects and other waste generated during maintenance are essential for overall facility hygiene. Training personnel on proper handling and care of the insects ensures the longevity and success of the insect culture. Through a comprehensive approach addressing these aspects, researchers can create an environment conducive to the study and cultivation of insects in glasshouse or protected cultivation settings (Cohen, 2018).

Contamination poses a substantial threat to the integrity and success of insect cultures, potentially emanating from diverse sources that include microbial intruders, parasitic organisms, cross-contamination, chemical pollutants, unwanted species introductions, and improper feeding practices. Microbial contamination, comprising bacteria, fungi, and viruses, can infiltrate cultures through contaminated substrates, food, or equipment, necessitating the strict implementation of sterile techniques and robust biosecurity measures (Inglis et al., 2009). Mites and parasitic infestations, if unchecked, can undermine the health and reproductive capabilities of the targeted insect species, necessitating vigilant monitoring and prompt isolation and treatment of infected individuals. Cross-contamination risks arise when equipment or personnel move between different insect cultures without proper cleaning, emphasizing the importance of designated equipment and stringent hygiene practices. Chemical contamination, arising from pesticides or cleaning agents, can have toxic effects on insect cultures, underscoring the need for careful storage and insect-friendly cleaning practices. Unwanted species introduction, a common challenge, demands rigorous quarantine measures for incoming insects to prevent resource competition, predation, or hybridization (Cohen, 2018).

The economic impact of fungus gnats (*Bradysiaspp.*) in greenhouses is significant, causing damage to young root systems, spreading soilborne diseases, and reducing crop marketability (Cloyd and Zaborski, 2004). Both adult and larval fungus gnats were found to indirectly transmit plant pathogens, including *Botrytis cinerea*, *Verticillium*, *Fusarium*, and *Thielaviopsis basicola*. And also, larvae feed on plant roots or tunnel into plant crowns, thus causing direct damage (Cloyd, 2008). So, this study was conducted to identify insect contaminants in the protected cultivation of cotton, tomato, and brinjal, which are reared for the purpose of maintaining whiteflies and aphid cultures.

MATERIAL AND METHODS

Theinsect culture of whiteflies, aphids, and mealybugs is maintained for research purposes, and the following study was conducted here for a period of two years from 2022–2023, Delhi. We observed the culture contamination by fly, which is black and predominantly in the culture of insects, especially in the summer season. So, we collected those insects. The gathered insects were carefully placed into containers with the intention of subsequently identifying the species. To ensure accurate identification, the collected specimens were meticulously compared against established reference samples and relevant literature (Menzel et al., 2019). For the purpose of molecular identification, samples of the fly were meticulously collected and carefully preserved in a solution of 70% ethanol, maintaining a stable temperature of 20 ºC until DNA extraction. The DNA extraction process was executed using a modified version of the CTAB method (Gouda et al., 2024a). The extracted DNA underwent evaluation through electrophoresis on a 0.8% agarose gel infused with 0.5 g/ml of ethidium bromide (Gouda et al., 2024b). The quantified DNA samples were then subjected to further analysis via PCR. Specifically, a fragment of the mtCOI gene was selectively amplified using the universal primers LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAACTTCAGGGTGACCAAAAAATCA-3'). In a reaction mixture of 25 µl, consisting of 12.5 µl of PCR master mix (Promega M750A), 7.5 µl of nuclease-free water, 1 µl each of forward and reverse primers, and 3 µl of the DNA template, PCR amplification was meticulously carried out (Gouda et al., 2024c). Subsequently, a portion (3 µl) of the PCR-amplified product was subjected to electrophoresis at 100 volts for a duration of 45 minutes on a 1.2% agarose gel in 1X TAE buffer (Gouda et al., 2024d). To ensure a thorough analysis, the purification and sequencing of the amplified PCR products were outsourced. Subsequently, a BLAST analysis was performed, utilizing the National Centre for Biotechnology Information (NCBI) as a valuable resource for the identification of homologous sequences (http://ncbi.nlm.nih.gov/BLAST). The resultant sequence was submitted to the NCBI GenBank to obtain the relevant accession numbers. For the purpose of conducting homology searches, multiple alignments were conducted using the Clustal W algorithm software (Gouda et al., 2024e). Furthermore, to enhance our understanding and visualize relationships, dendrograms were generated using the MEGA11 software. Reference strain sequences, pivotal for contextualizing our findings, were meticulously obtained from GenBank. This meticulous methodology was put in place to ensure the reliability and validity of the results obtained from the current study (Gouda and Subramanian, 2024).

RESULTS AND DISCUSSION

During our study, we found two species of fungus gnats, *Bradysiaocellaris* C. and *Bradysia impatiens* J. *B.ocellaris* and *B. impatiens*, closely related species of phorid flies in the family Phoridae, are commonly encountered in glasshouses and protected cultivation settings, posing challenges as potential contaminants of insect cultures (Fig. 1). While both species share similarities in size (typically 2-3 mm) and general morphology, accurate identification requires attention to key taxonomic characters. Antennae morphology is a critical differentiator. In *B. ocellaris*, the third antennal segment (club) is distinctly enlarged and oval-shaped, with a short arista (hair-like projection). In contrast, *B. impatiens* has a slightly enlarged and roundish third antennal segment with a longer arista. The scutellum, a dorsal plate on the thorax, provides another distinguishing feature. *B. ocellaris* has a scutellum with two pairs of bristles, while *B. impatiens* has three pairs of bristles (Mohrig et al., 2012).

Flight behavior variations also aid in identification, as *B. ocellaris* tends to hop or jump short distances, while *B. impatiens* exhibits more continuous flight. Additionally, their host preferences differ, with *B. ocellaris* having a

broader host range and *B. impatiens* showing a preference for fungal resourcesPoldmaa et al., 2015. These subtle morphological differences in antennae, scutellum, male genitalia, flight behavior, and host preferences collectively contribute to the taxonomic separation of *B. ocellaris* and *B. impatiens* in glasshouse and protected cultivation environments. To solidify the species identity beyond doubt, advanced molecular identification techniques were harnessed, utilizing the NCBI BLAST algorithm. The outcome of this analysis revealed an impressive 99.14% similarity match with *B.ocellaris*and 100% with *B. impatiens*, leading to the successful assignment of the obtained sequence with the accession numbers OQ706120 and OQ706121 (Fig. 2).

These insects were found to emerge from the soil of plants maintained for insect culture. They were more in pots with high moisture and also sub sequenced greenish algal growth. We observed that the larvae of these flies feed on fungi and organic debris, potentially competing with the target insects for food and reducing their nutritional intake, which is similar to the findings of Poldmaa et al., 2015. These fungus gnats were found to act as vectors for various pathogens, including bacteria and fungi, potentially transmitting them to reared insects and jeopardizing their health and viability, as observed by Cloyd, 2015. And also, their excreta were found to act as a substrate for the growth of sooty mold on plant leaves. Apart from this, large populations of fungus gnats caused a nuisance for facility workers and disrupted their research activities, as previously reported by Chu et al., 2004.

And we followed the following management practices to reduce its infestation: maintaining hygiene by establishing a clean and sanitary environment by regularly removing organic debris, old food sources, and damp substrates. Keep humidity levels slightly lower than optimal for your target insects, as fungus gnats thrive in moist conditions. By screening, we installed fine mesh screens on cages and ventilation openings to prevent adult gnats from entering the facility. Suitable substrates were incorporated that were less attractive to fungus gnats, such as sand or coconut coir, instead of peat moss or compost. Gnat traps were adapted to attract and trap adult gnats using yellow sticky traps. Apart from these physical controls, like drenching the soil with insecticides like spinosad directly on the soil and targeting larvae. Sand barriers were made of a thin layer of sand around the base of pots to deter larvae from moving to new substrates. Apart from these, quarantine measures like new insect arrivals should be kept in isolation for a period to ensure they are free of pests and diseases. Monitor populations by regularly inspecting insect cultures and breeding areas for signs of fungus gnats, including adult flies, larvae, and pupae. And also, regularly replace used substrates and clean breeding containers to disrupt pest life cycles.

In this study, the identification and management of *B.ocellaris* and *B. impatiens*, closely related fungus gnat species, were critical for preserving the integrity of insect cultures in glasshouses. Accurate taxonomic classification, relying on morphological characteristics and molecular techniques, was emphasized to distinguish between the two species. These fungus gnats, emerging from plant soil, exhibited larval feeding habits that competed with target insects for food resources, potentially compromising their nutritional intake. The study also revealed the role of these gnats as vectors for pathogens and contributors to the development of sooty mold on plant leaves. Implementation of rigorous management practices, including maintaining cleanliness, using physical controls like screens and traps, applying targeted insecticides, and enforcing quarantine measures, proved effective in mitigating infestations. The findings underscore the importance of a comprehensive approach to pest control in maintaining optimal conditions for insect cultures in controlled environments, offering valuable insights for future research and facility management.

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Fig. 1. Adult of a. *BradysiaOcellaris;* b. *Bradysia impatiens*

a. b.

Fig. 2. A phylogenetic tree illustrating the relationships among mtCOI sequences of fungus gnats constructed using the maximum likelihood approach in MEGA 11. Sequences generated in the present study are highlighted in red for clarity. This tree provides a visual representation of the genetic associations, aiding in the understanding of evolutionary patterns and genetic diversity within the fungus gnat populations.