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Fungal assemblages associated with gall formation by *Phytolyma fusca* on *Milicia excelsa* in Nigeria

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Abstract. *Olorunnibe VN, Omoloye AA, Alabi OY. 2025. Fungal assemblages associated with gall formation by* Phytolyma fusca *on* Milicia excelsa *in Nigeria. Cell Biol Dev 9: 91-98. Milicia excelsa* is an economically important tropical timber species whose cultivation is frequently constrained by gall formation induced by the iroko gall bug, *Phytolyma fusca*. Gall development modifies leaf tissues and may create microhabitats that facilitate fungal establishment, yet information on fungi associated with gall systems in *M. excelsa* under Nigerian nursery conditions remains limited. This study investigated fungal assemblages associated with gall formation by *P. fusca* on *M. excelsa* using conventional isolation and morphology-based identification techniques. Samples were collected from healthy leaves, ruptured and unruptured galled leaves, and from nymph and adult stages of *P. fusca*. Fungal isolation was conducted on potato dextrose agar, followed by purification and identification based on cultural and microscopic characteristics, and fungal occurrence was analyzed descriptively. Four fungal taxa were recovered exclusively from gall-associated tissues and insect stages: *Fusarium solani* (30%), *Fusarium oxysporum* (30%), *Aspergillus niger* (20%), and *Colletotrichum coccodes* (20%). No fungi were isolated from healthy leaves maintained under protected conditions, and fungal occurrence was restricted to gall-affected tissues and insect stages, with some taxa preferentially associated with ruptured galls. These findings demonstrate that insect-induced gall tissues function as localized microhabitats supporting distinct fungal assemblages, reflecting opportunistic rather than pathogenic colonization of modified plant tissues. This study provides baseline, non-pathogenic evidence of fungal assemblages restricted to *P. fusca*-induced gall microhabitats in *M. excelsa*. Further studies incorporating molecular identification and pathogenicity assays are required to clarify the ecological roles of the associated fungi.

Keywords: Fungal assemblages, gall formation, *Milicia excelsa*, nursery ecology, *Phytolyma fusca*

INTRODUCTION

Milicia excelsa is one of the most valuable indigenous timber tree species in tropical Africa and plays an important ecological and economic role in West African forestry systems. The species is widely cultivated for its high-quality hardwood, which is used for construction, furniture, and various industrial purposes (Ofori and Cobbinah 2007; Ugwu and Omoloye 2015). Despite its importance, the successful cultivation of *M. excelsa* in nurseries and plantations is often constrained by pest infestations and associated secondary biological stressors that reduce seedling vigor and survival rates. Among these constraints, gall-forming insects represent one of the most persistent challenges affecting young plants under plantation conditions.

The iroko gall bug, *Phytolyma fusca* (Hemiptera: Psyllidae), is widely recognized as a major pest of *M. excelsa* in Nigeria and other parts of West Africa (Ofori and Cobbinah 2007; Olajuyigbe et al. 2015; Ugwu et al. 2019). Feeding by *P. fusca* induces gall formation on leaves, resulting in abnormal tissue proliferation, distortion of leaf architecture, and impairment of normal physiological processes (Wagner et al. 2008). Severe infestations may lead to reduced photosynthetic capacity, premature leaf senescence, and increased susceptibility of affected tissues to secondary colonization by microorganisms. Thus, gall formation represents not only a direct insect-mediated stress but also a localized modification of plant tissues that may facilitate subsequent biological associations.

Plant galls are increasingly recognized as complex ecological niches rather than simple pathological structures. Gall tissues often provide relatively stable microclimatic conditions and altered nutrient availability that can support assemblages of microorganisms, including fungi and bacteria (Raman et al. 2012; Venkateswarlu et al. 2015). Several studies have documented the occurrence of fungal taxa within gall tissues of different plant species, suggesting that galls may function as entry points or reservoirs for opportunistic fungi following tissue disruption (Stone and Schönrogge 2003; Raman 2011). These fungi are frequently interpreted as secondary colonizers exploiting modified tissues rather than as primary causal agents of gall formation.

Fungal colonization of woody plants has long been associated with tissue injury, environmental stress, and insect activity. In *M. excelsa*, previous studies have reported fungal involvement in shoot dieback and seedling decline, with species such as *Lasiodiplodia theobromae* and *Fusarium solani* frequently isolated from diseased tissues (Apetorgbor et al. 2001). More broadly, fungi are known to enter host tissues through natural openings or wounds, including those created by insect feeding, thereby

establishing secondary associations that may exacerbate plant stress (Gaddeyya et al. 2012; Salau 2012). However, the presence of fungi in insect-induced galls does not necessarily imply pathogenicity, as many species may function primarily as saprophytes or opportunistic plant-associated fungi utilizing altered tissues.

Despite the recognized importance of *P. fusca* as a gall-forming pest of *M. excelsa*, information on fungal assemblages associated with gall tissues and insect developmental stages under Nigerian nursery conditions remains limited. Most available studies have focused either on the insect pest itself (Ugwu and Omoloye 2015; Ugwu et al. 2019) or on fungal pathogens causing overt disease symptoms (Apetorgbor et al. 2001), with little attention given to the broader fungal community occurring in gall-affected tissues. As a result, baseline ecological data on fungi associated specifically with insect-induced gall systems in *M. excelsa* are still lacking.

Accordingly, the present study was designed to address this knowledge gap by providing a descriptive, baseline assessment of fungal assemblages associated with gall tissues and insect stages of *P. fusca* on *M. excelsa* cultivated in Nigeria. Specifically, this study documents which culturable fungal taxa are associated with gall-affected tissues and insect developmental stages, and whether such fungi are absent from healthy, non-galled leaves under nursery conditions. This study does not test pathogenicity, vector relationships, or causal effects of fungi on gall formation or plant health; rather, it aims to characterize fungal occurrence as an ecological association linked to insect-induced tissue modification. By clarifying this scope, the study contributes foundational information for future work incorporating molecular identification, pathogenicity assays, and integrated pest and disease management strategies for sustainable *M. excelsa* cultivation. This study hypothesized that leaf galls induced by *P. fusca* on *M. excelsa* create localized microhabitats that selectively support culturable fungal assemblages absent from healthy leaf tissues, with differences in fungal occurrence between ruptured and unruptured galls.

MATERIALS AND METHODS

Study site and plant material

The study was conducted at the nursery facilities of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, southwestern Nigeria. The nursery is located within the humid tropical zone, characterized by relatively high annual rainfall and temperature conditions that are favorable for both plant growth and insect activity. All experimental procedures were carried out under routine nursery management practices commonly applied for raising *M. excelsa* seedlings in the region.

A total of twenty (n = 20) *M. excelsa* seedlings of similar age and growth stage were used as plant material for the study. The seedlings were raised in nursery containers under two contrasting exposure conditions. Ten seedlings were maintained inside a screened cage designed to exclude insect entry and served as non-galled plant material. The remaining ten seedlings were placed in an open nursery environment where natural infestation by *P. fusca* was allowed to occur, resulting in the formation of leaf galls.

Seedlings maintained in the open nursery were regularly inspected for gall development. Once galls were visibly formed, leaves exhibiting unruptured and ruptured galls were identified and selected for subsequent sampling. Seedlings kept under screened conditions did not develop galls and were used as sources of healthy leaf samples for comparative purposes. This experimental arrangement enabled the assessment of fungal assemblages associated with gall formation under natural infestation conditions while maintaining non-galled plant material as reference samples.

Sample collection

Samples were collected from both protected and naturally infested *M. excelsa* seedlings in the nursery. Five categories of samples were obtained to represent plant tissues and insect developmental stages associated with gall formation. These included healthy leaves collected from seedlings maintained inside the screened cage, unruptured galled leaves, ruptured galled leaves, nymphs of *P. fusca*, and adult insects collected from infested seedlings in the open nursery environment.

Healthy leaf samples were obtained from non-galled seedlings to serve as reference plant material. Galled leaves were carefully excised from infested seedlings and classified based on gall condition, namely unruptured galls and ruptured galls, to account for differences in tissue exposure. Nymphs and adult *P. fusca* individuals were collected directly from gall-affected leaves using sterilized forceps to minimize external contamination.

Immediately after collection, all samples were placed in sterile, labeled sample bottles to prevent cross-contamination among sample types. The samples were transported promptly to the Pathology Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, for further processing. During transportation, care was taken to maintain sample integrity by avoiding prolonged exposure to ambient conditions that could promote microbial growth prior to laboratory handling. All samples were processed within a short period after arrival at the laboratory to ensure that the isolated fungal assemblages reflected conditions present at the time of sampling.

Culture media preparation

Potato Dextrose Agar (PDA) was used as the culture medium for the isolation of fungal assemblages from plant tissues and insect samples. The medium was prepared by dissolving 39 g of commercially available PDA powder in 1000 mL of distilled water in a clean conical flask. The mixture was gently heated with continuous stirring until the medium was completely dissolved and homogeneous.

To minimize bacterial contamination during fungal isolation, streptomycin sulphate was used as a bacteriostatic agent. Prior to sterilization, the conical flask

containing the prepared medium was plugged with cotton wool and covered with aluminum foil. The medium was then sterilized by autoclaving at 121°C under standard pressure for 15 minutes. After autoclaving, the sterilized medium was allowed to cool gradually to approximately 45-47°C.

Once the medium had cooled to the appropriate temperature, a few drops of sterile streptomycin sulphate solution were aseptically added and gently mixed to ensure uniform distribution of the antibiotic. The medium was subsequently poured into sterile Petri dishes under aseptic conditions and allowed to solidify at room temperature. Prepared plates were left undisturbed until use for fungal isolation to prevent condensation and surface contamination.

Isolation and purification of fungi

Isolation of fungi from plant and insect samples was carried out using standard surface sterilization and culture techniques to minimize external contamination. All samples, including healthy leaves, ruptured and unruptured galled leaves, nymphs, and adult insects, were first cleaned by gently wiping their surfaces with sterile cotton wool. The samples were then surface-sterilized by immersion in 70% ethanol for approximately two minutes, followed by rinsing with sterile distilled water to remove residual alcohol.

After surface sterilization, plant samples were aseptically cut into small segments of approximately 2 mm in diameter using a sterile scalpel. Insect samples were processed whole due to their small size. Each sample segment or insect was aseptically placed onto the surface of solidified PDA plates prepared as described previously. Multiple segments from each sample category were plated to enhance the likelihood of fungal recovery. The inoculated Petri dishes were incubated at 25°C under ambient laboratory conditions for five days.

Fungal growth was monitored daily during the incubation period. Emerging fungal colonies were distinguished based on differences in colony color, texture, and growth pattern. Colonies exhibiting distinct morphological features were selected for further purification. To obtain pure cultures, hyphal tips from actively growing margins of individual colonies were aseptically transferred to fresh PDA plates using a sterile inoculating needle. The needle was flamed until red hot and allowed to cool before each transfer to prevent cross-contamination.

Subculturing was repeated several times until uniform colony morphology was observed, indicating successful purification. Pure cultures were maintained on PDA plates at room temperature and used for subsequent morphological and microscopic identification.

Morphological identification

Identification of fungal isolates was based on cultural and morphological characteristics following standard mycological procedures. Macroscopic features such as colony color, texture, margin characteristics, growth rate, and pigmentation on PDA were recorded for each isolate after incubation. These characteristics were used to distinguish among fungal taxa and to guide preliminary identification at the genus and species level.

Microscopic examination was conducted using the lactophenol cotton blue staining technique. A small portion of aerial mycelium from actively growing cultures was mounted on a clean glass slide in a drop of lactophenol cotton blue and covered with a cover slip. The mounted preparations were examined under a light microscope using 10× and 40× objective lenses. Microscopic features, including hyphal structure, septation, conidiophore morphology, and conidial shape and arrangement, were observed and compared with standard identification keys and published descriptions.

Species identification in this study was based solely on morphological criteria and therefore represents putative identification. No molecular or DNA-based techniques were employed, and taxonomic assignments should be interpreted within the limitations of morphology-based identification.

Data analysis

Data obtained from fungal isolation were analyzed using descriptive statistics. The frequency of occurrence of each fungal taxon was calculated based on the number of isolates recovered from all sample types. Percentage occurrence was determined by expressing the frequency of each isolate as a proportion of the total number of fungal isolates recovered. It should be noted that the number of seedlings examined in this study was limited, and the fungal assemblages reported represent only culturable fungi recovered under the applied sampling intensity. Furthermore, because fungal isolation was conducted exclusively on potato dextrose agar, the results reflect fungi capable of growth on this medium and should not be interpreted as representing the total fungal diversity associated with gall tissues or insect stages of *P. fusca*.

RESULTS AND DISCUSSION

Fungal taxa recovered from gall-associated samples

A total of four fungal taxa were recovered from gall-associated plant tissues and insect samples of *P. fusca* on *M. excelsa*. The isolated fungi comprised *F. solani*, *F. oxysporum*, *Aspergillus niger* and *Colletotrichum coccodes*. These taxa were consistently obtained from galled leaf tissues and insect developmental stages but were absent from healthy leaf samples collected from seedlings maintained under screened conditions.

No fungal growth was observed from healthy leaves throughout the incubation period, indicating the absence of culturable fungi under the conditions applied in this study. In contrast, fungal colonies emerged readily from ruptured and unruptured galled leaves as well as from nymphs and adult insects collected from naturally infested seedlings. The occurrence of fungal taxa varied among sample types, with some fungi being recovered from multiple sources, including plant tissues and insect stages.

Fusarium solani was isolated from nymphs, adult insects, and unruptured galled leaves, whereas *F. oxysporum* was recovered from both ruptured and unruptured galled leaves as well as from nymphs and adult insects. *Aspergillus niger* was isolated exclusively from ruptured galled leaves, while *C. coccodes* was recovered from ruptured galled leaves and adult insects. The distribution of fungal taxa across sample categories highlights variation in fungal assemblages associated with different gall conditions and insect developmental stages.

The cultural and microscopic characteristics used for fungal identification are presented in Table 1, whereas the frequency and percentage occurrence of each fungal taxon across all samples are summarized in Table 2. Collectively, these results indicate that fungal assemblages were restricted to gall-affected tissues and insect stages, with no fungi detected in non-galled plant material under the conditions of this study.

Morphological characteristics of fungal isolates

The fungal isolates recovered from gall-associated tissues and insect stages of *P. fusca* exhibited distinct cultural and microscopic characteristics that facilitated their morphological differentiation (Table 1). Colony appearance on potato dextrose agar varied among taxa in terms of growth rate, texture, pigmentation, and surface morphology.

Isolates identified as *F. solani* produced rapidly expanding colonies that were woolly to cottony in texture, with abundant white to cream aerial mycelium and a cream-colored reverse. Microscopically, these isolates displayed hyaline, septate hyphae and characteristic conidiophores bearing conidia consistent with descriptions of the genus *Fusarium*. The isolates were recovered from unruptured galled leaves as well as from nymphs and adult insects.

Colonies of *Fusarium oxysporum* were also fast-growing but differed from *F. solani* in their relatively paler pigmentation and smoother colony surface. Microscopic examination revealed septate hyphae and the presence of chlamydospores occurring either singly or in chains, which are diagnostic features commonly associated with *F. oxysporum*. This taxon was isolated from both ruptured and unruptured galled leaves as well as from insect samples.

Aspergillus niger isolates formed dense, felt-like colonies with dark green to black pigmentation on PDA. The colonies were compact in appearance and showed limited aerial mycelial extension compared with *Fusarium* species. Microscopic observation revealed erect conidiophores terminating in globose conidial heads with radiating chains of conidia, a morphology typical of *A. niger*. These isolates were obtained exclusively from ruptured galled leaves.

Colonies of *C. coccodes* exhibited a grayish-white, cottony appearance with a relatively compact and thickened texture. Under microscopic examination, the isolates produced unicellular, hyaline, cylindrical conidia, often showing a slight constriction at the midpoint. This species was recovered from ruptured galled leaves and adult insects. Collectively, the observed morphological characteristics supported the differentiation of the four fungal taxa recovered in this study.

Frequency of occurrence of fungal assemblages

The frequency and percentage occurrence of fungal taxa recovered from gall-associated tissues and insect samples of *P. fusca* are summarized in Table 2. A total of four fungal taxa were isolated, with notable variation in their frequency of occurrence across all samples examined.

Fusarium solani and *F. oxysporum* were the most frequently recovered taxa, each accounting for six isolates, corresponding to 30% of the total fungal assemblage. Together, these two taxa constituted 60% of all fungal isolates obtained in the study, indicating their widespread association with gall-affected tissues and insect stages. Both species were recovered from multiple sample types, including galled leaves and insects, reflecting a broader distribution within the sampled system.

Table 2. Frequency and percentage occurrence of fungal isolates recovered from *Milicia excelsa* and *Phytolyma fusca*

Fungal taxon	Frequency	Percentage occurrence (%)
Fusarium solani	6	30
Colletotrichum coccodes	4	20
Aspergillus niger	4	20
Fusarium oxysporum	6	30

Table 1. Morphological and microscopic characteristics of fungal isolates recovered from gall-associated tissues of *Milicia excelsa* and developmental stages of *Phytolyma fusca*

Fungal isolate	Host part	Colony appearance on PDA	Microscopic characteristics
Fusarium solani	Nymph, adult, unruptured galled leaf	Rapid-growing colonies, woolly to cottony; white to cream aerial mycelium with cream-colored reverse	Hyaline, septate hyphae; presence of conidiophores and conidia typical of *Fusarium* spp.
Fusarium oxysporum	Nymph, adult, ruptured and unruptured galled leaf	Fast-growing colonies, pale to lightly pigmented	Septate hyphae with chlamydospores formed singly or in chains
Aspergillus niger	Ruptured galled leaf	Dense, felt-like colonies, dark green to black in color	Erect conidiophores with globose conidial heads and radiating conidia
Colletotrichum coccodes	Ruptured galled leaf, adult	Grayish-white, cottony colonies with compact texture	Unicellular, hyaline, cylindrical conidia, often slightly constricted

Note: Species identification based on cultural and microscopic morphology

In contrast, *A. niger* and *C. coccodes* each accounted for four isolates, representing 20% of the total fungal assemblage, respectively. These taxa were less frequently recovered than *Fusarium* species and exhibited a more restricted distribution across sample categories. *Aspergillus niger* was isolated only from ruptured galled leaves, whereas *C. coccodes* was recovered from ruptured galled leaves and adult insects.

The observed differences in frequency among fungal taxa highlight variation in their relative occurrence within gall-associated environments. While *Fusarium* species dominated the assemblage, other taxa contributed to the overall fungal diversity associated with gall formation. Importantly, no fungal isolates were recovered from healthy leaf samples, underscoring the restriction of fungal occurrence to gall-affected tissues and insect stages under the conditions applied in this study.

Discussion

Gall tissues as niches for fungal assemblages

The results of this study demonstrate that fungal assemblages were consistently associated with gall-affected tissues and insect stages of *P. fusca*, while no fungi were recovered from healthy leaves. This pattern supports the view that insect-induced galls represent distinct microenvironments that differ markedly from surrounding healthy plant tissues. Gall formation involves localized tissue proliferation, structural modification, and altered physiological processes, which together create conditions that may favor microbial establishment (Wagner et al. 2008; Raman et al. 2012).

Plant galls are increasingly regarded as specialized ecological niches rather than simple pathological abnormalities. The enclosed structure of galls can provide relatively stable microclimatic conditions, including moderated humidity and altered nutrient availability derived from modified host metabolism (Stone and Schönrogge 2003; Raman 2011). Such conditions may explain why fungal taxa were detected exclusively in gall-affected tissues in this study and were absent from intact, healthy leaves of *M. excelsa*.

Comparative studies from other gall systems support this interpretation. Fungal associations have been documented in galls induced by various insect groups across a wide range of host plants (Raman et al. 2012; Venkateswarlu et al. 2015). In these systems, fungi are commonly reported from gall tissues following mechanical damage or physiological alteration caused by galling insects, often exploiting modified tissues rather than acting as primary pathogens (Carroll 1988; Arnold et al. 2003).

In the *M. excelsa*-*P. fusca* system examined here, gall tissues appear to provide suitable substrates for fungal establishment, particularly when galls rupture and expose internal tissues. The recovery of *A. niger* and *C. coccodes* predominantly from ruptured galls suggests that tissue exposure may further facilitate fungal presence. Similar patterns have been observed in other woody plants, where fungal occurrence increases following gall rupture or tissue necrosis (Parke et al. 2007). In contrast, unruptured galls may function as semi-closed structures that restrict colonization to taxa capable of establishing under more limited exposure.

The dominance of *Fusarium* species within the assemblage is consistent with their broad ecological distribution and known association with stressed or altered plant tissues (Apetorgbor et al. 2001; Gaddeyya et al. 2012). Their frequent recovery from gall tissues and insect stages reflects ecological compatibility with gall-modified environments rather than evidence of pathogenic activity.

The findings indicate that gall tissues induced by *P. fusca* on *M. excelsa* function as localized niches supporting distinct fungal assemblages associated with tissue modification and exposure. Importantly, the observed patterns represent ecological associations between fungi, insect-induced galls, and plant tissues, and should be interpreted as associative rather than causal evidence of fungal involvement in gall formation or plant disease.

Association of Fusarium *spp. with gall-affected tissues*

Among the fungal assemblages recovered in this study, *F. solani* and *F. oxysporum* were consistently isolated from multiple gall-associated sample types, including ruptured and unruptured galled leaves as well as nymphs and adult stages of *P. fusca* (Table 1). In terms of overall frequency, each taxon accounted for 30% of the total isolates recovered across all samples (Table 2). Their repeated occurrence across different components of the gall-insect system suggests a consistent ecological association with gall-affected tissues rather than incidental contamination.

Species of *Fusarium* are widely distributed in terrestrial ecosystems and are commonly reported as components of plant-associated fungal communities. They are frequently isolated from roots, stems, leaves, and soil, particularly in environments where plant tissues are stressed or physically damaged (Gaddeyya et al. 2012; Mailafiya et al. 2017). In woody plants, *Fusarium* spp. have often been detected in association with tissue wounds, insect feeding sites, and senescing organs, where they may act as saprophytes or weak opportunists rather than primary pathogens (Nelson et al. 1983; Leslie and Summerell 2006).

In the context of *M. excelsa*, previous studies have reported the isolation of *F. solani* from seedlings exhibiting dieback symptoms (Apetorgbor et al. 2001). However, those studies focused on visibly diseased plants and did not specifically address the role of gall formation or insect activity in facilitating fungal presence. In contrast, the present study documented the occurrence of *Fusarium* spp. specifically in gall-affected tissues and insect stages, while healthy leaves remained free of culturable fungi. This pattern suggests that gall formation and associated tissue modification may create favorable conditions for *Fusarium* colonization.

The ability of *Fusarium* species to colonize gall tissues may be attributed to their ecological versatility. *Fusarium solani* and *F. oxysporum* are known to tolerate a wide range of environmental conditions and can rapidly exploit nutrient-rich substrates (Aoki et al. 2014). Gall tissues, characterized by altered cellular organization and localized accumulation of nutrients, may therefore provide suitable microhabitats for these fungi. The recovery of *Fusarium*

spp. from both ruptured and unruptured galls indicates that colonization may occur at different stages of gall development, although the mechanisms underlying entry and establishment remain unclear.

Importantly, while *Fusarium* species are often associated with plant diseases, their presence alone does not confirm pathogenic activity. Many *Fusarium* taxa exhibit endophytic or saprophytic lifestyles depending on host condition and environmental context (Kuldau and Yates 2000; Arnold et al. 2003). In the absence of pathogenicity assays or molecular confirmation, the isolates recovered in this study should be interpreted as components of a fungal assemblage associated with gall-affected tissues rather than as definitive causal agents of disease.

The association of *Fusarium* spp. with insect stages of *P. fusca* further suggests close spatial proximity between fungi and the galling insect. Similar associations have been reported in other plant-insect systems, where fungi are recovered from insects inhabiting or feeding within modified plant tissues (Vega and Dowd 2005). Such associations may reflect passive acquisition of fungal propagules from the gall environment rather than active transmission or vectoring by the insect.

The frequent recovery of *F. solani* and *F. oxysporum* from gall-associated tissues highlights their prominence within the fungal assemblage linked to *P. fusca* infestations on *M. excelsa*. These findings emphasize the need to view *Fusarium* spp. within this system as ecologically associated fungi whose roles may range from opportunistic colonization to potential contributors to tissue degradation under specific conditions. Further studies employing molecular identification and pathogenicity testing are required to clarify their functional significance within gall-affected *M. excelsa* tissues.

Opportunistic colonization following tissue damage

In addition to *Fusarium* species, the fungal assemblages associated with gall-affected tissues of *M. excelsa* included *A. niger* and *C. coccodes*, which were predominantly isolated from ruptured galled leaves and, in the case of *C. coccodes*, also from adult insects (Table 1). Both taxa were recovered at lower frequencies compared with *Fusarium* species (Table 2). Their restricted occurrence in ruptured galls indicates a closer association with exposed or damaged tissues than with intact gall structures.

Aspergillus niger is a cosmopolitan fungus commonly found in soil, plant debris, and decomposing organic matter, and is widely recognized for its saprophytic lifestyle and ability to exploit substrates rich in readily available nutrients (Toma et al. 2021). In plant systems, *A. niger* is frequently reported from necrotic or senescing tissues and is typically regarded as a secondary colonizer rather than a primary pathogen (Samson et al. 2014). Its exclusive recovery from ruptured galls in this study is therefore consistent with colonization following tissue exposure rather than with an active role in gall formation.

Similarly, *C. coccodes* was isolated from ruptured galled leaves and adult insects but was absent from unruptured galls and healthy leaves (Table 1). Species of *Colletotrichum* are known to exhibit diverse ecological strategies, ranging from pathogenic to endophytic and saprophytic lifestyles depending on host condition and environmental context (Cannon et al. 2012). In several plant systems, *Colletotrichum* spp. have been reported to persist asymptomatically or to colonize damaged tissues following mechanical injury or senescence (Peres et al. 2005). The occurrence of *C. coccodes* in ruptured galls therefore suggests opportunistic establishment facilitated by tissue exposure rather than a causal role in tissue damage.

The association of these fungi with ruptured gall tissues highlights the importance of physical tissue disruption in shaping fungal assemblages. Gall rupture represents a transition point at which previously enclosed tissues become exposed to external environmental conditions, increasing contact with airborne fungal propagules. Such exposure provides access to newly available substrates that can be exploited by saprophytic or weakly associated fungi, as reported in other gall systems (Parke et al. 2007; Raman 2011).

The recovery of *C. coccodes* from adult *P. fusca* further indicates spatial overlap between insects and fungi within gall-affected environments. However, this co-occurrence should not be interpreted as evidence of active fungal transmission by the insect. Instead, insects inhabiting ruptured galls may passively acquire fungal propagules from colonized tissues, reflecting shared microhabitats rather than vector-mediated processes (Vega and Dowd 2005).

The presence of *A. niger* and *C. coccodes* in gall-affected tissues of *M. excelsa* is best interpreted as an ecological association linked to tissue damage and exposure. The evidence presented here is associative rather than causal and does not support a primary pathogenic or vector-mediated role of these fungi in gall formation or plant disease.

Insect-fungus-plant interaction: Ecological perspective

The association between gall-forming insects, plant tissues, and fungi represents a complex ecological interaction that does not necessarily imply direct transmission or vectoring of microorganisms by insects. In the *M. excelsa-P. fusca* system examined in this study, fungal taxa were recovered from gall tissues and insect stages, but such co-occurrence should be interpreted within a framework of indirect ecological association rather than active insect-mediated transmission.

Gall formation by *P. fusca* fundamentally alters host plant tissues through localized cell proliferation, disruption of normal tissue organization, and modification of nutrient allocation (Wagner et al. 2008). These changes create microhabitats that may be favorable for microbial establishment, particularly fungi that are capable of exploiting stressed or modified tissues. In this context, the insect acts as an ecosystem engineer by creating gall structures that indirectly facilitate fungal colonization, rather than as a biological vector introducing fungi into plant tissues (Stone et al. 2001; Raman 2011).

The recovery of fungal taxa from both gall tissues and insect developmental stages suggests spatial proximity between insects and fungi within gall environments. Insects inhabiting galls are continuously in contact with plant tissues that may already harbor fungal propagules. As a result, fungal spores or hyphal fragments may adhere passively to insect bodies without implying a functional role in fungal dispersal or infection (Vega and Dowd 2005). Similar passive associations have been documented in other insect-plant systems, where fungi are recovered from insects occupying confined or nutrient-rich microhabitats (Six 2012).

Importantly, the distinction between association and vectoring is critical when interpreting insect-fungus interactions. Vector relationships typically involve specific adaptations that facilitate pathogen acquisition, retention, and transmission, often accompanied by consistent disease outcomes in host plants. Such evidence was not generated in the present study, which relied on morphological identification and descriptive analysis. Consequently, the fungal taxa documented here should be regarded as associated organisms that coexist within gall-affected environments rather than as pathogens actively transmitted by *P. fusca*.

From an ecological perspective, gall systems represent multi-trophic interactions involving plants, insects, and microorganisms. The presence of fungi within these systems may influence gall microenvironments by contributing to tissue degradation, nutrient cycling, or competitive interactions with other microorganisms (Arnold et al. 2003; Six 2012). However, the functional roles of fungi within gall tissues remain poorly understood and are likely context-dependent, varying with gall developmental stage, tissue condition, and environmental factors.

The findings of this study support an interpretation of insect-fungus-plant interactions based on indirect ecological associations. Gall formation by *P. fusca* modifies plant tissues in ways that create opportunities for fungal colonization, while fungi and insects co-occur within shared microhabitats without clear evidence of vector-mediated processes. Recognizing this distinction is essential for accurately interpreting fungal occurrence in gall systems and for avoiding overestimation of causal relationships in plant health assessments.

Methodological limitations and future directions

The findings of this study should be interpreted in light of several methodological limitations inherent to the experimental design and analytical approach. First, fungal identification was based exclusively on cultural and morphological characteristics. While morphology-based identification remains a widely used and valuable approach in mycology, particularly for preliminary surveys, it has well-recognized limitations in resolving species boundaries, especially within taxonomically complex genera such as *Fusarium* and *Colletotrichum* (Leslie and Summerell 2006; Cannon et al. 2012). Morphological similarity among closely related species may result in ambiguous or putative identifications, and cryptic species diversity cannot be ruled out without molecular confirmation.

Second, the absence of molecular analyses, such as Internal Transcribed Spacer (ITS) sequencing or multilocus approaches, limits the taxonomic resolution of the isolates recovered in this study. DNA-based identification has become the standard for accurate fungal taxonomy and would enable more robust comparisons with global datasets (Schoch et al. 2012; O'Donnell et al. 2015). Future studies should incorporate molecular tools to confirm species identities and to explore phylogenetic relationships among gall-associated fungal taxa in *M. excelsa* systems.

Another important limitation concerns the lack of pathogenicity testing. The present study did not include inoculation experiments or Koch's postulates to assess the ability of isolated fungi to cause disease symptoms in *M. excelsa*. As a result, no conclusions can be drawn regarding the pathogenic roles of the fungi recovered. This limitation is particularly relevant for taxa such as *Fusarium* spp., which are known to exhibit diverse ecological strategies ranging from saprophytic to pathogenic lifestyles depending on host and environmental conditions (Kuldau and Yates 2000). Future research should include controlled pathogenicity assays to distinguish between opportunistic colonizers and potential disease agents.

The study also relied on a relatively limited sample size and was conducted under nursery conditions at a single location. Although this approach provided valuable baseline information, broader spatial and temporal sampling would improve understanding of variability in fungal assemblages associated with gall formation. Comparative studies across multiple nurseries, plantation sites, and seasons would help clarify the consistency of observed patterns and the influence of environmental factors on fungal occurrence.

Despite these limitations, the present study provides an important foundation for future investigations into fungal assemblages associated with gall systems in tropical woody plants. Building on these findings, future research should integrate molecular identification, pathogenicity testing, and ecological analyses to elucidate functional roles of fungi within gall environments. Such approaches would contribute to a more comprehensive understanding of plant-insect-fungus interactions and support the development of integrated management strategies for *M. excelsa* cultivation.

In conclusion, this study documented fungal assemblages associated with gall formation by *P. fusca* on *M. excelsa* under nursery conditions in Nigeria. Four fungal taxa, namely *F. solani*, *F. oxysporum*, *A. niger*, and *C. coccodes*, were isolated from gall-affected leaf tissues and insect developmental stages, while no fungi were recovered from healthy leaves maintained under protected conditions. *Fusarium solani* and *F. oxysporum* were the most frequent taxa, each accounting for 30% of total isolates, together comprising 60% of the recovered assemblage. In contrast, *A. niger* and *C. coccodes* each represented 20% of isolates and were predominantly associated with ruptured galls, indicating that tissue exposure enhances fungal colonization. The restriction of fungal occurrence to gall-

associated environments highlights the role of gall formation in creating microhabitats conducive to fungal colonization. The predominance of *Fusarium* species within the assemblage, together with the occurrence of *Aspergillus* and *Colletotrichum* taxa in ruptured galls, suggests that tissue modification and exposure are important factors shaping fungal presence. These fungi are best interpreted as opportunistic or plant-associated taxa exploiting altered tissues rather than as confirmed causal agents of disease. As such, the findings provide baseline ecological information on fungi co-occurring with gall systems in *M. excelsa*. From a management perspective, the results underscore the importance of integrated approaches to *M. excelsa* cultivation that prioritize effective control of gall-forming insects while maintaining nursery hygiene to reduce secondary fungal colonization. Overall, this study provides essential baseline ecological evidence for understanding fungal co-occurrence within insect-induced gall systems of *M. excelsa*.

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