Studies on the Pathogenesis of the Local Isolates of *Nomuraea rileyi* against *Spodoptera litura*

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ABSTRACT: The mode of infection of *Nomuraea rileyi* in *Spodoptera litura* larvae was examined using local strains isolated from infected larvae of *S. litura* collected from the field in Mae Chaem district, Chiang Mai province, Thailand. Spore suspensions were topically applied to larvae followed by examination using standard histological techniques. Germ tubes penetrated through the cuticle surface within 48 hr after inoculation and then along the cuticle. Lysis of the endocuticle was found before hyphae penetrated and invaded the epidermis ca. 2.5 days after inoculation. The invasive hyphal stage then transformed into hyphal bodies that replicated within the hemocoel. These non-invasive hyphal bodies filled the hemocoel and then converted to invasive mycelia ca. 5-6 days after inoculation. At the end of the infection cycle, mycelium emerged from the cuticle and produced condiophores ca. 7 days after inoculation. The larvae became completely covered by white mycelium before turning green 1-2 days later as a result of spore production. The complete developmental cycle of *N. rileyi* in *S. litura* lasted approximately 8 to 9 days.

List of abbreviations: bc, blood cell; Cu, cuticle; D, dermal cell; ed, endocuticle; ep, epicuticle; epd, epidermis cell; ex, exocuticle; FMAY, fish-soluble medium supplement with 1% maltose, 1% yeast extract and 1.5% agar; hb, hyphal body; hp, hyphae; m, muscle; μm, micron; nu, nucleus.

KEYWORDS: *Spodoptera litura*, *Nomuraea rileyi*, pathogenesis and bioinsecticide.

INTRODUCTION

The tobacco cutworm *Spodoptera litura* is regarded as a major destructive pest of subtropical and tropical agricultural crops. This noctuid is distributed throughout the Kingdom of Thailand. Control currently relies mainly on the application of various classes of chemical insecticides including carbamates, pyrethroids and organophosphates.[1] It is recognized that widespread continuous use of these chemical insecticides causes environmental problems and leads to the development of insect resistance. Microbial insecticides such as entomopathogenic fungi can provide an alternative, more environmentally friendly option to control this insect pest.

The fungus, *Nomuraea rileyi* is a dimorphic hyphomycete that can cause epizootic death in various insects. It has been shown that many insect species belonging to *Lepidoptera* including *S. litura* and some belonging to *Coleoptera* are susceptible to *N. rileyi*.[2] The host specificity of *N. rileyi* and its eco-friendly nature encourage its use in insect pest management. Although, its mode of infection and development have been reported for several insect hosts such as

Trichoplusia ni[3], *Heliothis zea*[4], *Plathypena scabra*[5], *Bombyx mori*[6], *Pseudoplusia includens*[7] and *Anticarsia gemmatalis*[8], there is no similar report for *S. litura*. This prompted us to study the pathogenesis of *N. rileyi* against *S. litura* using a fungal isolate from Mae Chaem that was identified as entomopathogenic *N. rileyi* based upon morphological and physiological characteristics[9]. The aim was to assess the possibility of using the isolate as a biocontrol agent. The mode of infection of the fungus in *S. litura* was studied employing histopathological techniques.

MATERIALS AND METHODS

Source of Fungus

Local isolates of entomopathogenic fungi were isolated from diseased larvae in the field at Amphoe Mae Chaem, Chiang Mai province in the northern part of Thailand, during December 2000. Pure cultures were established and maintained in 3% fish-soluble extract, by-product of canned tuna industry, supplement with 1% maltose, 1% yeast extract and 1.5% agar (FMAY).

Source and Multiplication of Insect Larvae

The culture of *S. litura* was raised from field-collected larvae and maintained under laboratory conditions. Eggs laid on the surface of cabbage leaves by adult females were allowed to hatch and develop to 1st instar larvae, after which they were fed with castor leaves in chambers until they reached the pupal stage. The pupae were collected and used as a stock to build up new colonies. Adult insects were maintained in cages and fed with 10% sugar solution.

Preparation of Conidial Suspensions

Conidial suspensions were prepared by diluting the fresh sporulated cultures with 0.02% tween 80 in sterile distilled water plus 0.05% spreader stick agent, alkylphenol ethoxylate and glycols (free fatty acids and isopropanol). Test insects were treated with conidial suspension at a concentration of 10^8 conidia/ml.

Larval Treatment

The late 2nd instar larvae of *S. litura* were topically challenged with 5 ml of conidial suspension at a concentration of 10^8 conidia/ml and then placed in a growth chamber regulated at 25 °C with 95 % relative humidity. They were fed with castor leaves. The test larvae were observed every 6 to 12 hr until death followed by white mycelial growth on the surface of the cadavers.

Histopathological Methods

The infected larvae collected every 6 to 12 hr were fixed in Bouin's fluid for 24 hr. They were subsequently washed with several changes of 70 % ethanol in order to remove the fixative agent. Dehydration was done through a series of graded ethanol, at concentrations of 70%, 80%, 90%, 95% and 100% (v/v), for 15 min each. Then, they were embedded in paraffin wax for sectioning at 5 μm thickness. Sections were subsequently deparaffinized and stained with Harris's Hematoxylin and Eosin dye, mounted, and examined under the light microscope (Olympus, model BX50).

RESULTS

The growth and development of *N. rileyi* in *S. litura* was studied histologically, in comparison to untreated larvae (Fig. 1). Germ tube penetration through the insect cuticle was first observed by 2 days after inoculation. It occurred without appressorium formation. Instead, the germ tubes grew along the endocuticle between the epidermis and the exocuticle laminae and produced lateral branches (Fig. 2). Lysis of the endocuticle occurred before hyphae invaded the epidermis. The hyphae penetrated the epidermis and reached the hemocoel within two and a half days
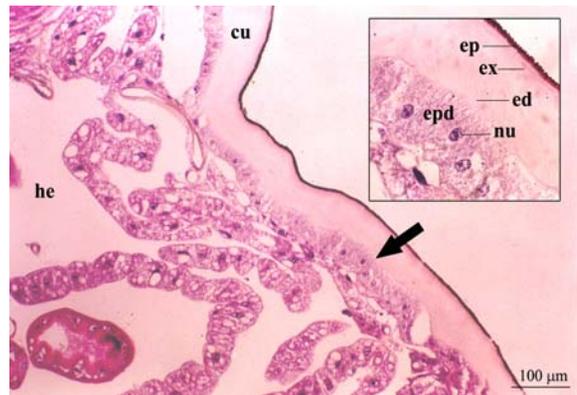

Fig 1. Longitudinal section of non-infected larva of *S. litura* showing the cuticular layers (Cu) and epidermal cells (epd) at a magnification of 400x. (Inset = a picture at 2.5x higher magnification, of the area around the arrow).
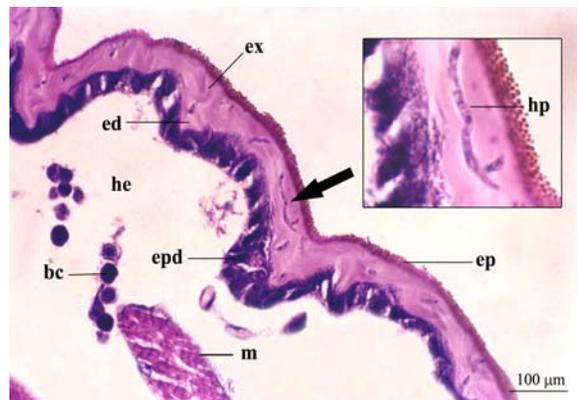

Fig 2. Longitudinal section of infected *S. litura* larva 2 days after *N. rileyi* inoculation. The picture shows penetrant hyphae (hp) that developed from germ tubes and propagated along the endocuticle laminae (ed) between exocuticle (ex) and epidermis (ep) at a magnification of 400x. (Inset = a picture at 2.5x higher magnification, of the area around the arrow).
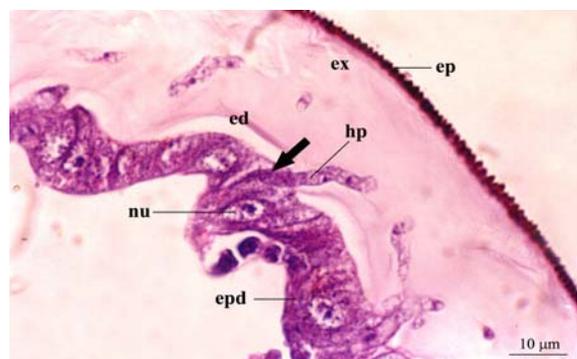

Fig 3. Two and a half days after *N. rileyi*, the hyphae penetrated the epidermis and grew into the hemocoel. Arrow shows the point of penetration where lysis of endocuticle occurred (1000x).

after inoculation (Fig. 3). After reaching the hemocoel, the invasive hyphae transformed into hyphal bodies that replicated in the hemolymph by budding (Fig. 4). Noninvasive hyphal bodies filled the hemocoel in a few days and all tissues became colonized (Fig. 5). Nevertheless, these processes did not affect larval feeding activity or digestion and they appeared to be grossly healthy. The noninvasive hyphal bodies converted to invasive mycelia at 5.5 to 6 days after inoculation, completely ramified throughout all larval tissues, penetrated the cuticle and emerged through it (Fig. 6). However, the emerging hyphae differed from the hyphae found in the early stages of infection in that they grew perpendicular to the cuticular surface rather than parallel to it (compare Figs. 2 and 6). The hyphae emerged from the cuticle of the dead larva and grew all over the surface forming a white mycelial mat. They usually died with the anterior portion of their body in an elevated position. Newly-formed conidia were visible on conidiophores at 7.5 days after inoculation.
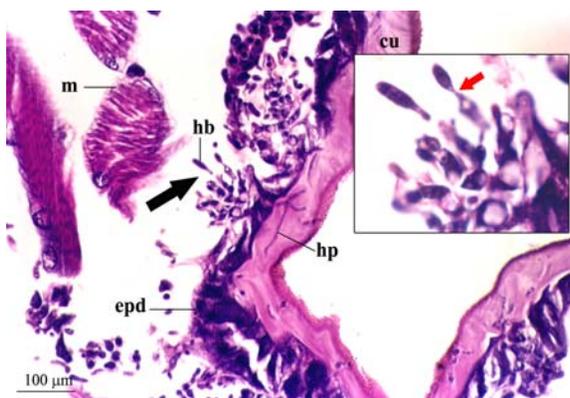

Fig 4. Invasive hyphae transformed into hyphal bodies and replicated by budding and septum formation in the hemocoel by 3 days after *N. rileyi* inoculation (400x).
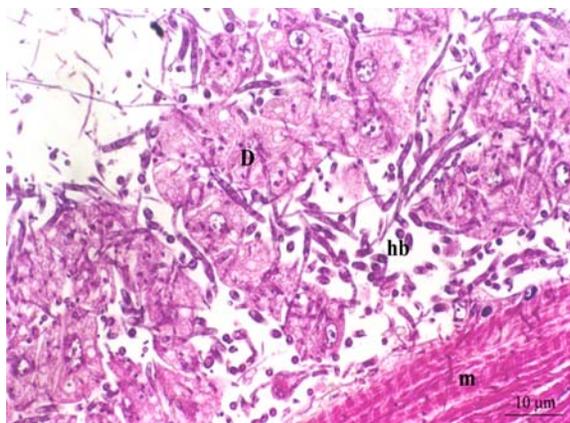

Fig 5. All tissues of infected larvae were colonized with hyphal bodies by 5.5 to 6 days after *N. rileyi* inoculation (1000x).
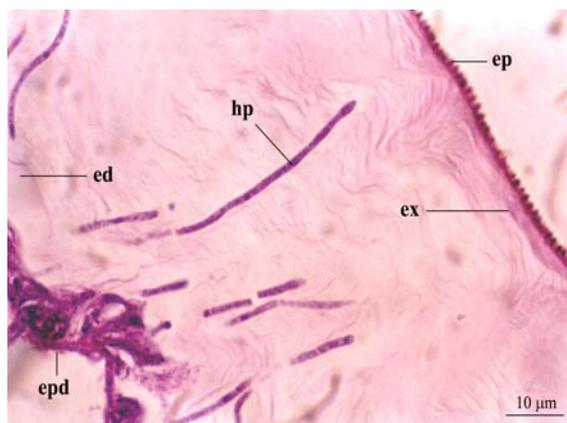

Fig 6. At 6.5 days after *N. rileyi* inoculation, the non-invasive hyphal bodies converted to invasive mycelia and penetrated through cuticle (1000x).

Subsequently, cadavers became completely covered by green conidia. The complete developmental cycle of this *N. rileyi* isolate in *S. litura* lasted approximately 8 to 9 days.

DISCUSSION

The germination periods for *N. rileyi* conidia vary with host. Germination of the conidia on the integument of *S. litura* (46 to 48 hr) was similar to that reported for *Heliothis zea*[4] and on *Spodoptera frugiperda*[10] but slower than that reported for *Pseudoplusia includens* (less than 8 hr)[7] and *Anticarsia gemmatalis* (6 to 18 hr).[8] These differences in time could be attributed to the variation in the composition of the integument of different larvae. Germination of *N. rileyi* conidia was induced by the addition of larval cuticle and yeast extract to minimal medium.[11] The penetration of entomopathogenic fungi through the cuticle is sometimes preceded by the formation of an appressorium that firmly attaches to the epicuticle and provides the fulcrum for the mechanical and enzymatic processes that mediate penetration.[12] However, appressorium formation was not detected in this study, suggesting that penetration by *N. rileyi* was primarily enzymatic rather than mechanical processes. The detection of endocuticular cell lysis prior to the invasion of hyphae into the epidermis was similar to phenomena described by Getzin[3] and Mohamed.[4] By contrast, Boucias[8] and Thorvilson[5] did not find lysis of the endocuticle.

Once the fungal isolate had successfully penetrated into the hemocoel and transformed into hyphal bodies, there was no recognition by hemolymph opsonins or hemocyte (blood cell) surface receptors and no phagocytosis by circulating hemocytes.[13] It is possible that this resulted because of similarity between surface

components of *N. rileyi* and insect cells allowing the hyphal bodies to evade a host immune response.[14] In contrast, hyphal bodies of *Beauveria bassiana*, *Paecilomyces fumosoroseus*, and *Paeciomyces farinosus* are immediately recognized and phagocytized by insect granulocytes and plasmatocytes.[15] The depletion of nutrients and water or the expression of specific cellular components may activate the conversion of hyphal bodies back to tissue-invasive hyphae.[16] The complete developmental cycle of *N. rileyi* isolate in *S. litura* lasted ca. 8 to 9 days and closely paralleled the course of its growth in other noctuid larvae. Finally, this study will be useful for the development of *N. rileyi* as a bioinsecticide. Nevertheless, detailed studies under field conditions are necessary before releasing *N. rileyi* for the control of *S. litura* in nature.

Acknowledgements

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