CONIDIAL DISCHARGE OF AN ENTOMOPATHOGENIC FUNGUS INFECTING THE COTTON LEAFHOPPER¹

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ABSTRACT

A laboratory experiment was conducted to determine the conidial discharge of *Batkoa amrascae* Keller & Villacarlos, an entomopathogenic fungus found infecting the cotton leafhopper on okra. Field and laboratory-infected leafhopper cadavers were tested for the effect of light. The fungus from field-infected cadavers kept in the dark during sporulation produced an average of 26,372 conidia (29 hr. mean time conidial production), whereas those exposed to light had 5,290 conidia (33 hr. mean time conidial production). On the other hand, laboratory-infected cadavers exposed to light attained an average of 4,447 conidia while that of cadavers kept in the dark had 3,373 conidia, both had 28 hr mean time conidial production.

KEY WORDS: Conidial discharge. Entomopathogenic fungi. *Batkoa amrascae*. Biological control. Cotton leafhopper. *Amrasca biguttula*.

¹ Part of M.S. thesis of the author.

INTRODUCTION

Several pests have been reported to attack okra (*Abelmoschus esculentus* L. Moench) among which the cotton leafhopper, *Amrasca biguttula* Ishida, is the most important (Peter and David, 1988). The cotton leafhopper usually inhabits the nether surface of the leaves, hence it is difficult to control heavy infestations even with weekly or twice-weekly application of insecticides. The quick growth and frequent harvest of fruits also preclude the possibility of using insecticides for control due to residue problems (Uthamasamy, 1986). Hence, the possible use of other control alternatives is imperative.

An entomopathogenic fungus, *Batkoa amrascae* Keller and Villacarlos, has been identified as a potential biological control agent of cotton leafhopper attacking okra. In only a few days, *B. amrascae* is observed to destroy the okra leafhopper population in the field.

No research has been conducted in the Philippines on the pathological, biological and ecological characteristics of this entomopathogenic fungus. Considering its potential as a biological control agent, this study was conducted to gather information on conidial discharge and sporulation from individual okra leafhopper cadavers and determine the effect of light exposure on conidial production.

METHODOLOGY

Mass Rearing of Cotton Leafhoppers

Healthy adult leafhoppers collected from the field were placed inside a 61 x 122 x 56 cm screen cage and were provided with 1-month old potted okra plants. Adults were allowed to mate until enough insects of known age were available.

Source of Infected Insects

Field-infected leafhopper cadavers

Field-infected adult leafhoppers that had not yet sporulated were carefully removed from the okra leaf substrate where they get attached by the fungal rhizoid. The cadavers were placed in a sterile petri plate, air-dried for 2-3 days and kept dry inside the refrigerator prior to their use in the experiment.

Laboratory-infected leafhopper cadavers

Several field-infected leafhopper cadavers were used to infect healthy leafhoppers under laboratory condition. Okra leaf discs (4.3 mm in diameter) were placed individually in screw cap vials (11.3 mm in length, 4.5 mm in diameter) bottom-lined with moist filter paper prior to introduction of healthy leafhoppers. Ten (10) healthy adult leafhoppers were removed from the rearing cage using a Macaulay battery-operated respirator and transferred into screw cap vials. For each vial, five (5) leafhoppers were placed inside together with one field-infected cadaver. The cadaver was attached to the center of a clean glass slide using a small portion of water agar. The slide was placed upside down on top of the mouth of the vial containing the insects. Sporulating

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cadavers were allowed to shower conidia on the healthy leafhopper adults for 15 hours. Dead leafhopper adults were then collected and air-dried.

Laboratory Set-up on Conidial Discharge

Ten (10) newly field-infected leafhopper adults and another batch of 10 laboratory infected insects that had not yet sporulated were used in the study. The cadavers were placed singly in the middle of the petri plate lined with moistened filter paper. A glass slide supported with a rubber band (about 2 mm thickness) was placed on top of the cadaver to catch the forcibly discharged conidia. Every 30 minutes, the exposed glass slide was replaced with a clean one until all the conidia were properly labelled based on the time it was removed above the sporulating cadaver. Each slide was flooded with lactophenol cotton blue, covered with a cover slip and examined under the microscope. The number of conidia collected on the slides was counted using a compound microscope. To determine if there was any effect of light on conidiation, the first batch of 5 cadavers (either field of laboratory infected) were exposed to continuous light using two 40-watts fluorescent bulb while the remaining 5 were kept in the dark. The release of conidia was monitored every 30 min. Light intensity during conduct of the study was determined using a light meter.

RESULTS AND DISCUSSION

Conidial Production of Field-Infected Leafhopper Cadavers

The number of discharged conidia from cadavers exposed to light and those produced in the dark varied greatly depending on the individuals (Fig. 1). Cadavers from both set-up started initial conidiation

Figure 1. Average number of *Batkoa amrascae* conidia discharged per hour from field-infected cadavers of cotton leafhoppers with and without light.

after an average of 2.5 hr exposure in the moisture chamber. An average of 26,372 conidia were counted from 5 cadavers without light source (mean time conidial production of 29 hr) compared to 5,290 conidia obtained with light source (mean time conidial production of 33 hr). A series of distinct conidial peak patterns were also observed in both set-up. The highest conidiation peak (without light source) was observed 7 hr after exposure to high humidity chamber followed by 2 lower peaks 10 and 12 hr thereafter. A rapid decline on intensity of conidial production was then recorded ending after 31 hr. On the other hand, highest peak for conidial production of cadavers (with light source) occurred 9 hr after, followed by a slightly lower peak at 13 and 21 hr. Lower conidial counts were detected until conidiation terminated after 34 hr, ending relatively 3 hr longer than cadavers without light source. Thus, cadavers exposed to light source had very low conidial production and ended their conidiation for a longer time compared to cadavers unexposed to a light source. Apparently, the degree of epizootic occurrence, host and pathogen population, and the weather conditions in the area where and when the cadavers were collected had an effect on conidial production aside from the light requirements of the entomopathogen. The presence of primary, secondary and tertiary conidia were also observed projected by the cadavers. Primary conidia can be differentiated from secondary and tertiary conidia generally by their smaller size.

Several workers have attempted to characterize patterns of conidial discharge. The emergence of conidiophores of Entomophthorales usually occurs within a few hours of host death and discharge of conidia soon commences, (MacLeod, 1963). Cohn (1985) as cited by Mullens and Rodriguez (1985) noted that

conidial discharge of *Entomophthora muscae*, for example, began sometime after 8 hr postmortem (HPM) and that the cadaver remained swollen for a few days. Gustaffson (1965) further noted abundant conidiation at 30 hr HPM in one muscoid fly. On the other hand, Carruthers (1982) observed that conidiation began in the field within a few hr of death, was most intense at 0100 hr, and was 95% complete by 0500 hr. Brobyn and Wilding (1983) reported that conidial germination occurred between 2 and 24 hr after discharge.

It was further observed that if the fungus failed to contact a host, primary conidia of many species had the ability to form and forcibly discharge secondary conidia and tertiary (subsequent) conidia until fungal resources were exhausted. In the field, the hosts generally succumbed during the afternoon and early evening hours, and production of primary conidia was found to be most intense at night, particularly between 2400 and 0600 hours (Newman and Carner, 1974; Aoki, 1981; and Carruthers, 1982). This is generally the period of highest humidity, an important factor in many *Entomophthorales* (MacLeod 1963, Yendol, 1968, Wilding, 1969, and Millstein et al., 1983).

Conidial Production of Laboratory-Infected Leafhopper Cadavers

Compared to field-infected cadavers particularly those devoid of light source, conidial production in cadavers in the laboratory yielded very low counts (Fig. 2). Initial sporulation began 8 hr for unexposed cadavers and 10 hr for light-exposed ones after exposure to moisture, respectively. A slightly lower average conidial counts of 3,737 was observed from 5 cadavers without light source. The first highest peak occurred 15 hr then followed at 2 hr after. Cadavers exposed to

Figure 2. Average number of *Batkoa amracae* conidia discharge per hour from laboratory-infected cadavers of cotton leafhoppers with and without light source.

light source yielded a slightly higher average conidial count of 4,447 until sporulation stopped. A series of conidial peaks was also observed in this set-up. The highest peak was observed 19 hr after exposure to moisture. Sporulation ceased after 37 hr while conidiation of cadavers under dark condition ended 2 hr earlier. A small but significant shift in mean time of conidial production from cadavers aside from conidial counts in the two set-ups was notable. Such differences may indicate some alteration of the fungus due to difference in laboratory conditions as opposed to that in the field. Variability of light requirements in fungal sporulation had been reported in many species (Cochrane, 1958, as cited by Alexopoulos and Mims, 1979). It was further added that light plays an important role in spore dispersal since spore-bearing organs of many fungi are positively phototropic and discharge their spores towards the light.

CONCLUSION AND RECOMMENDATION

Laboratory study on conidial discharge of cadavers showed higher average of 26,372 conidia from field-infected cadavers without light source (mean time conidial production of 29 hr) compared to only 5,290 conidia (meantime conidial production of 33 hr) for cadavers exposed to light source. On the other hand, conidial production of laboratory-infected cadavers was less intense. An average of 4,447 and 3,737 conidia were counted from cadavers exposed to light and without light source, respectively.

Results of this study provide basic information on the effect of light and duration of conidial production of field-infected or laboratory-infected cadavers. This is important in the manipulation of field population if ever *Batkoa amascae* is used as a biological control agent.

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