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CELLULAR EVENTS OCCURING DURING THE PATHOGENESIS OF ASPERGILLOSIS OF *GORGONIA* SPECIES

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ABSTRACT

Aspergillosis of *Gorgonia* spp. has been reported throughout the Caribbean and continues to be active in the Bahamas. The fungus responsible is probably a newly discovered species in the genus *Aspergillus*. Microscopic observations of affected colonies at different stages of infection were made and isolation of the pathogen from different sea fan structures revealed that the infection could progress at different rates among infected colonies. The response of the sea fan to infection also varied ranging from recession and necrosis of large areas of coenenchyme to production of pigmented sclerites which, in some cases, may limit the extent of the infection. This, in turn, appears to lead to the development of either flat or round galls which may sequester the advance of the pathogen. Studies are continuing to determine both the biological and chemical nature of the gorgonian response to *Aspergillus*.

INTRODUCTION

Sea fan mortalities were reported along the Central and South American coastlines in the 1980's (Guzman and Cortes, 1984; Garzon-Ferreira and Zea, 1992) . During this epizootic, complete mass mortality of *Gorgonia* spp. was observed in certain areas and recovery has not yet taken place (Cortes, 1997, personal communication). In the mid-1990's, sea fans exhibiting similar symptoms were first observed in Saba, then throughout the Caribbean (Nagelkerken *et al.*, 1997a) . Although the distribution of the disease was more extensive, partial, rather than complete mortality of infected colonies was observed during the more recent outbreak. Samples of infected tissue were obtained from various laboratories throughout the Caribbean via the CARICOMP network.

Microbiological analysis of diseased samples revealed the presence of the fungus *Aspergillus* which was not found in healthy tissue. These fungal isolates were shown to produce the disease symptoms when inoculated onto healthy seafans under axenic conditions (Smith *et al.*, 1996). These isolates were also found to be metabolically and genetically similar to each other.

The purpose of this paper was to describe the infection process at the cellular and tissue level based on microscopic observations of diseased tissue from throughout the Caribbean.

MATERIALS AND METHODS

Coral samples of healthy and diseased tissue were obtained from the Bahamas, British Virgin Islands, Curacao, Florida, Saba and Trinadad (Negelkerken *et al.*, 1997a,b). Microscopic observations of infected tissue were compared with healthy tissue and the progression of the infection was observed on healthy seafans which had been inoculated with pure cultures of the *Aspergillus* isolate. All cultures were grown on HE medium made up in 3.2% seawater (Smith *et al.*, 1996). Pure cultures were identified by extracting DNA, amplifying the rRNA region using the polymerase chain reaction with a universal eucaryotic primer and sequencing on an automated sequencer..

Transfection experiments were performed to test the transmissability of the disease. Sample squares (approx. 5 x 5cm of tissue) were removed from 20 healthy and 20 diseased *G. ventalina* colonies. Two tissue squares (one healthy and one diseased) were inserted into 10 healthy *G. ventalina* and 10 healthy *G. flabellum* colonies. After one month (from Nov. to Dec. 1995), the transfected colonies were observed for disease symptoms.

Galls were removed from Bahamian *G. ventalina*, surface sterilized in a 1.25% solution of

hypochlorite, cut in halves using autoclaved hacksaw blades and plated on HE medium.

RESULTS

Initial microscopic comparisons of healthy tissue with infected tissue revealed the presence of filamentous structures within diseased coenenchyme (Fig. 1). Although reproductive structures were not seen in the tissue, diseased tissue samples plated on solid media gave rise to fungi which produced conidiospores (Fig. 2). These fungi did not grow from healthy tissue. Coenenchyme receded in affected sea fans and the fungal hyphae were observed along the receding edge. As the coenenchyme receded, the axial skeleton was exposed. The fungus did not appear to degrade the axial skeleton, but the axis was often colonized by cyanobacteria and algae which, in some cases, did break down skeletal tissue.

Fungi isolated from diseased sea fans at all Caribbean sites were identified as *Aspergillus*. Blast searches of the GenBank and EMBL databases showed these isolates to be most similar to *Aspergillus fumagatus* (98%) but nucleotide sequences were more similar among the sea fan isolates than to any sequence in the rRNA gene database.

Recession of the living tissue and secondary colonization of the exposed skeletal tissue could be observed on sea fans *in situ* on reefs in San Salvador, Bahamas (Fig. 3), although the degree of infection (% of the colony affected) and the overall progression of the disease varied considerably among sea fan colonies. In most cases, purpling of the coenenchyme was observed both macroscopically (Fig. 3, below the secondary colonization at the top) and microscopically (Fig. 4). Microscopic observations of affected tissue indicated that the color change was due to the presence of pigmented sclerites.

Transfection experiments showed the the infection from *G. ventalina* could be transferred to other *G. ventalina* and *G. flabellum* colonies. The infection rate for *G. ventalina* was 60% (6 of 10) when diseased tissue was grafted into healthy colonies (Fig. 5) and 40% (4 of 10) for *G. flabellum*. Controls did not result in an active infection. Purpling of the recipient tissue and gall formation resulted in all infected tissue (regardless of active infection development), including some of the controls.

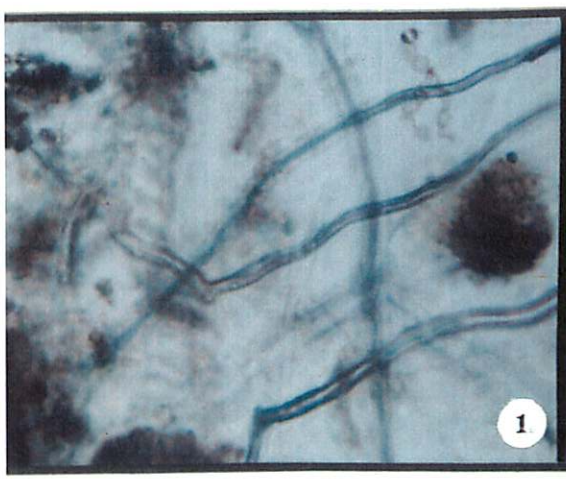
Galls (often referred to as 'agal tumors') were found on both sea fan species (Fig.6). Gall tissue was heavily pigmented and contained no polyps (Fig. 7). Because of the similarity between galls formed during the transfections and galls found on otherwise healthy colonies, surface sterilized galls were cut in half and plated on HE medium to determine if this could be a response to fungal infection. Within a week abundant hyphal growth was seen from the cut side of the galls (Fig. 8). Subcultures had typical *Aspergillus* morphology and rRNA gene sequencing confirmed the identity of the fungus.

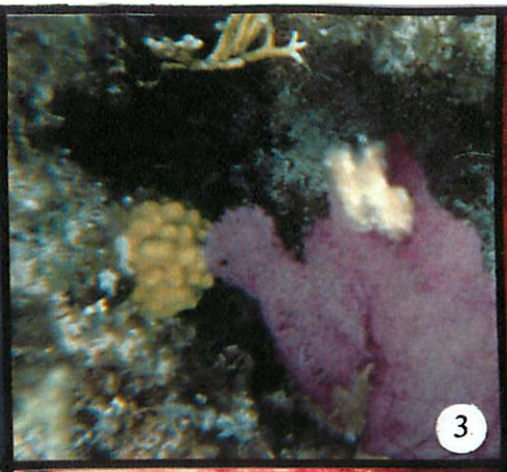
DISCUSSION

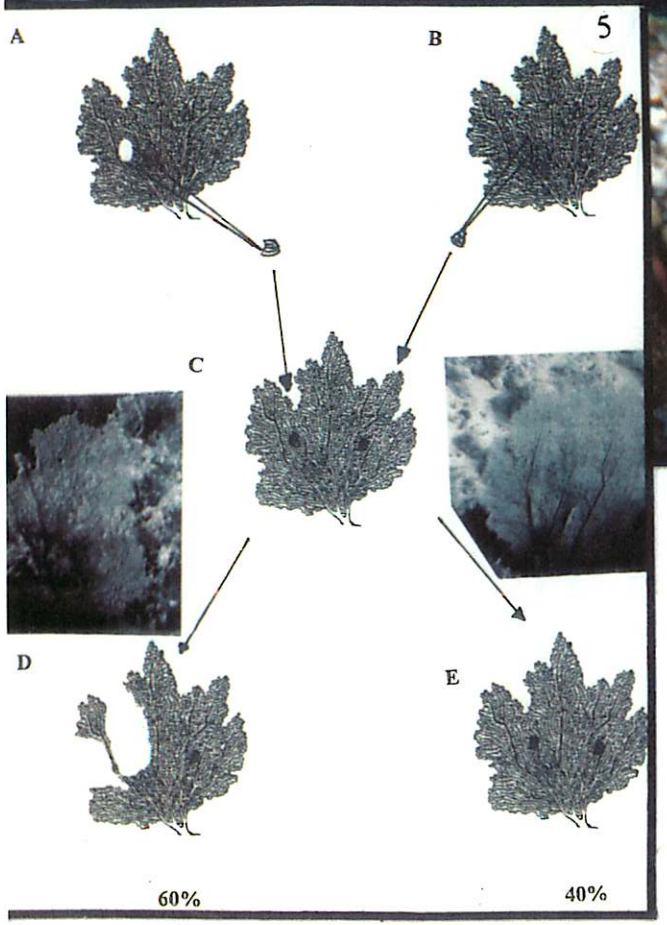
Fungi in the genus *Aspergillus* seem to be the cause of the current Caribbean epizootic. *Aspergillus* hyphae were found embedded in the coenenchyme at the receding edge of sea fans from all sites sampled. Pure cultures of the fungus were able to cause the infection in sterile aquaria (Smith *et al.*, 1996) and in seawater aquaria (Smith and Ritchie, unpub.) The fungi do not appear to sporulate while in contact with the sea fan tissue, but do when subcultured on defined media. It is not known if these organisms were also responsible for the mass mortalities of Caribbean sea fans in the 1980's (Guzman and Cortes, 1984; Garzon-Ferreira and Zea, 1992, Laydoo, 1983), but the symptomology of the past and present epizootics appear identical.

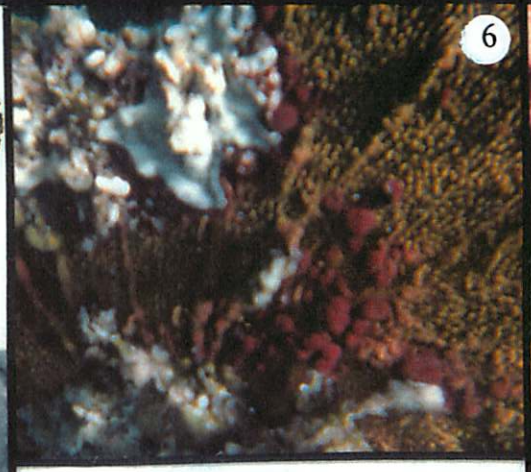
One response to the initial fungal infection appeared to be the darkening, or purpling, of the coenenchyme. This response appeared to be due to an increased concentration of pigmented sclerites in affected areas. Studies are in progress to quantitate this relationship. One hypothesis may be that an immediate, effective recruitment of sclerites to an infected area may serve to sequester the spread of the fungus by forming a physical barrier (in the form of galls) between the fungus and healthy tissue. This process would be analogous to abscess formation in animals or callus formation in plants. Even if this is the case, observations of diseased sea fans indicate the this response is only partially effective, since recession of the tissue often progresses past the purpling area. However, this may partly explain why only 60% of the *G. ventalina* colonies and 40% of the *G. flabellum* colonies became infected during the transfection experiments.

Evidence supporting the sequestering hypothesis come from the fact that *Aspergillus* was isolated from cut, surface-sterilized galls. The round galls have been referred to as 'algal tumors' by Morse *et al.* (1977,1981) who observed and characterized algal structures in the galls. It is possible that during gall formation other organisms (secondary invaders on axial skeletons) become incorporated into the gall. It is also possible that the sea fan gall formation response is nonspecific and may initiated by a number of invading









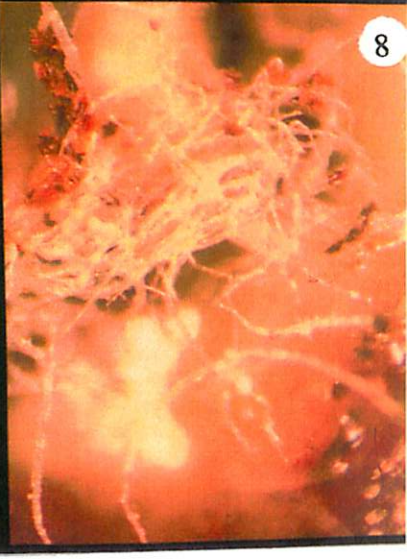


Figure 1. Coenenchyme tissue from diseased *G. ventalina* showing hyphae along the receding edge. **Figure 2.** Reproductive structures produced by infected sea fan tissue. **Figure 3.** Infected sea fan from San Salvador, Bahamas. Secondary colonization can be seen at the top of the colony, followed by the receding tissue (clear area). Photo by E. Brill. **Figure 4.** Photomicrograph of the surface of *G. ventalina* showing darkening of the tissue due to pigmented sclerites. **Figure 5.** Transfection experiment with *G. ventalina.* A- Infected donor tissue, B- Healthy donor tissue. C- Recipient healthy colony. D- 60% developed infection. E- 40% remained healthy **Figure 6**. Galls on *G. ventalina*. **Figure 7**. Photomicrograph of gall growing from normal tissue. **Figure 8.** Fungal hyphae growing from cut gall. The gall was obtained from otherwise healthy tissue.

organisms. Work continues to attempt to understand these processes.

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LITERATURE CITED

Garzón-Ferreira J, Zea S (1992) A mass mortality of *Gorgonia ventalina* (Cnidaria: Gorgoniidae) in the Santa Marta area, Caribbean coast of Colombia. Bull Mar Sci 50:522-526

Guzmán HM, Cortés J (1984) Mortandad de *Gorgonia flabellum* Linnaeus (Octocorallia: Gorgoniidae) en la Costa Caribe de Costa Rica. Rev Biol Trop 32:305-308

Laydoo R (1983) Recent mass mortality of gorgonians in Trinidad. Unpubl report. Institute of Marine Affairs, Trinidad, W.I.

Morse DE, Morse ANC, Duncan H (1977) Algal "tumors" in the Caribbean sea-fan, *Gorgonia ventalina*. Proc 3rd Int Coral Reef Symp 1:623-629

Morse DE, Morse A, Duncan H, Trench RK (1981) Algal tumors in the Caribbean octocorallian, *Gorgonia ventalina*: II. Biochemical characterization of the algae, and first epidemiological observations. Bull Mar Sci 31:399-409

Nagelkerken I, Buchan K, Smith GW, Bonair K, Bush P, Garzón-Ferreira J, Botero L, Gayle P, Heberer C, Petrovic C, Pors L, Yoshioka P (1997) Widespread disease in Caribbean sea fans: I. Spreading and general characteristics. Proc 8th Int Coral Reef Symp 1: 679-682.

Nagelkerken I, Buchan K, Smith GW, Bonair K, Bush P, Garzón-Ferreira J, Botero L, Gayle P, Harvell CD, Heberer C, Kim K, Petrovic C, Pors L, Yoshioka P (1997) Widespread disease in Caribbean sea fans: II. Patterns of infection and tissue loss. Mar. Ecol. Prog. Ser. (in press)

Smith GW, Ives LD, Nagelkerken IA, Ritchie KB (1996) Caribbean sea fan mortalities. Nature 383:487