

## Response of sea fans to infection with *Aspergillus* sp. (Fungi)

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**Abstract:** Mass mortalities of sea fans (*Gorgonia* spp.) off the South and Central American coasts were reported in the 1980s. In some cases, populations never recovered. Widespread tissue mortality in *G. ventalina* and *G. flabellum* throughout the Caribbean was reported in 1997. Although colony mortality was apparently lower in the more recent epizootic, the geographic distribution of the disease appeared much greater. Tissue samples of affected colonies were found to be infected with a fungus (*Aspergillus* sp.) which was shown to be able to cause the disease in healthy sea fans. We describe observations on the early infection process and subsequent development of the disease. Based on these observations, we report the response of *Gorgonia* spp. to infection by *Aspergillus*.

**Key words:** *Gorgonia*, sea fans, *Aspergillus*, disease.

Mass mortalities of sea fans were reported in the 1980s (Guzmán and Cortés 1984, Garzón-Ferreira and Zea 1992). These reports described almost complete mortality of sea fan populations from Costa Rica and Santa Marta (Columbia) within a short time (1993). The authors reported tissue losses up to 25% of the total living sea fan blade per week and observed complete mortality in certain sites in Trinidad (Laydoo 1983). Recently, beginning in 1995, another epizootic of sea fan disease was observed to occur throughout the Caribbean (Nagelkerken *et al.* 1997). Although more widespread, this recent epizootic, does not appear to be as virulent. Smith *et al.* (1996) isolated the fungus *Aspergillus* sp. from diseased tissue from Curaçoa, Saba, Trinidad, the British Virgin Islands, the Florida Keys, and the Bahamas. Transmissibility in the field was shown to occur when diseased sea fan tissue was grafted onto healthy sea fans. Furthermore,

the Saba fungal isolate was shown to be able to initiate disease signs on healthy sea fans when inoculated in pure culture in aquaria (Smith *et al.* 1996). In this communication, we describe aspects of the pathogenesis of *Aspergillus* sp. infection on *Gorgonia* spp., as revealed by microscopy.

In all diseased sample thus far observed, infections appear to begin by the recession of rind tissue (coenenchyme), exposing the axial skeleton (central core). Tissue removed from the receding edge was infested with fungal hyphae. These hyphae were not found in tissue samples collected from healthy sea fans (Fig. 1). Diseased tissue, when plated on artificial media, gave rise to fungal colonies. These were identified as belonging to the genus *Aspergillus* (Smith *et al.* 1996). The response of sea fans to infection was found to be varied. Often, initial response involved purpling of the tissue due to pigmented sclerites (Fig. 2).

Samples removed from healthy and diseased sea fans were analyzed for pigmented sclerite content. Diseased tissue exhibited a higher population of pigmented sclerites compared with healthy tissue (Fig. 3). Field observations of sea fans exhibiting disease signs revealed a variety of responses. Those colonies that showed little sclerite response demonstrated the greatest amount of mortality within the colony. Increases in pigmented sclerite concentration, in some cases, took place soon after disease signs appeared, and often resulted in the formation of galls (Fig. 4).

It would appear that the recruitment of sclerites is a defense mechanism which could protect the colony from the spread of infection.

The advance of the fungal hyphae may become sequestered by the formation of the galls. To examine whether *Aspergillus* was actually encased within galls, galled tissue was collected from the Bahamas and surface sterilized in a sodium hypochlorite solution (5.2%). These galls were split by sawing with a sterile hacksaw blade. Sections were then plated onto an artificial sea water medium. Within a week fungal hyphae appeared, growing from the gall (Fig. 5). These fungi were morphologically similar to the *Aspergillus* obtained from the receding rind tissue of the diseased sea fans. The identification of this fungus is presently being confirmed by rDNA sequence analysis. Recent studies indicate gall formation is pri-

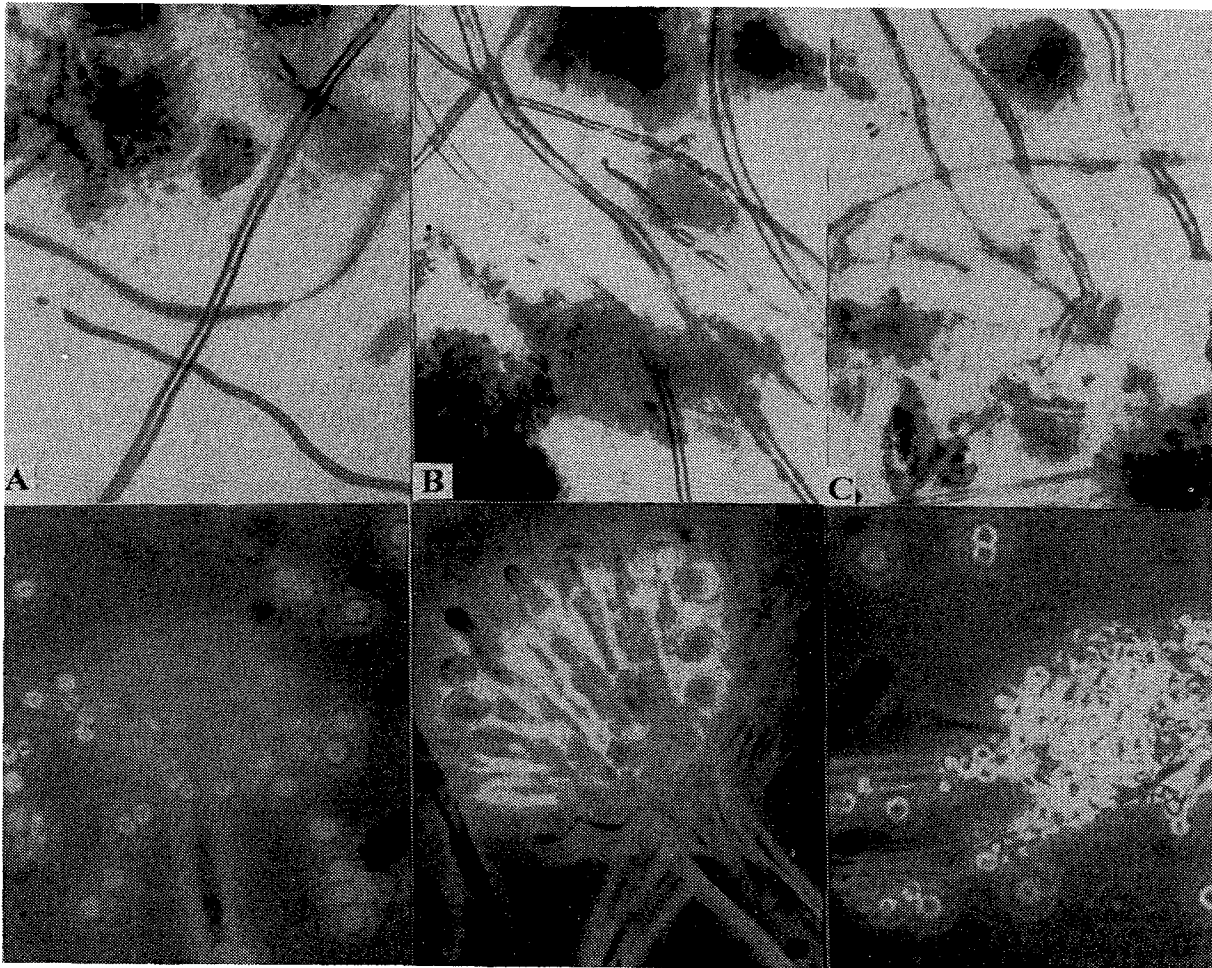


Fig. 1. Fungal hyphae from the receding edge (upper) of infected tissue from Saba (A), Bahamas (B) and Trinidad (C). Fruiting structures from infected tissue which was plated on a selective medium (lower).

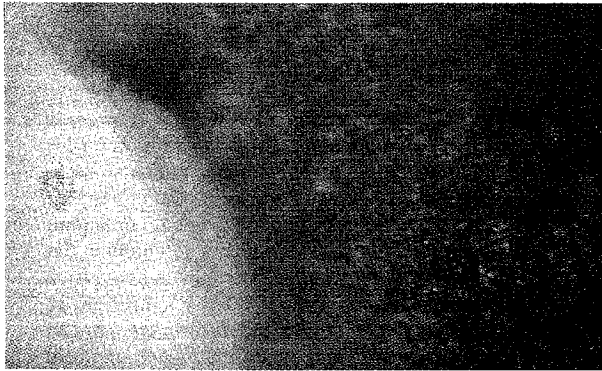


Fig. 2. Photomicrograph of the purpling of *G. ventalina* due to higher concentrations of pigmented sclerites (100X).

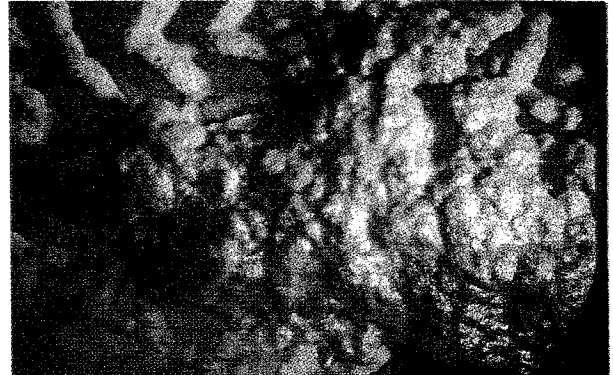


Fig. 4. Gall formation on *G. ventalina*.

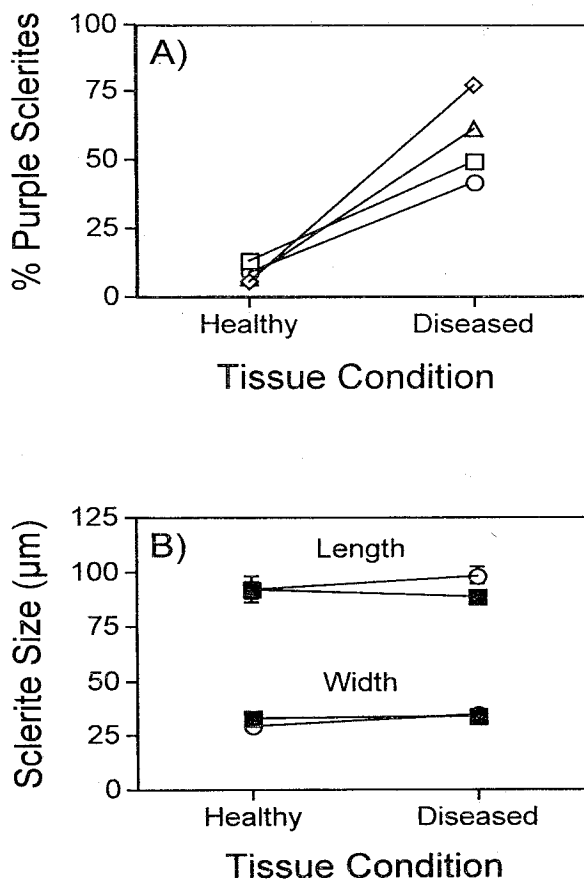


Fig. 3. Infection results with an increase in the production of purple sclerites; however, there appears to be little change in sclerite sizes. Proportion of purple sclerites and sclerite size were determined by counting and measuring the first 50 sclerites encountered under a compound microscope. A) Counts from samples collected in diseased and healthy areas from the same colony are connected by a line (n=4 colonies). B) Data represent means  $\pm$  standard errors of spindle sclerite lengths (n=4).

marily due to the increased deposition of gorgonin which could sequester the spread of the fungus as well as any secondary invaders. We have observed both viable *Aspergillus* and algae in some split galls. Much more work needs to be done before a definitive initiator of gall formation is determined.

The presence of the disease has been monitored in the Bahamas since 1995. Monitoring was done at five different sites using 2 by 50 meter belt transects. The incidence of the disease varied depending on the site monitored (Fig. 6) and has increased to the present by 5 to 30% (not shown). *Gorgonia ventalina* appears more susceptible to the disease than does *Gorgonia flabellum* (Fig. 6). In these surveys, all sea fans exhibiting signs of the disease were counted as diseased sea fans. Some of these sea fans may actually exhibit signs of past infections. Therefore, we may be counting active as well as arrested infections.

In summary, the present epizootic of sea fans in the Caribbean appears more widespread

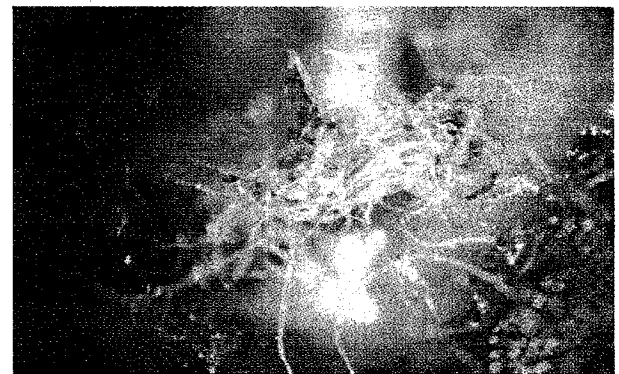


Fig. 5. Fungal hyphae growing from split gall.

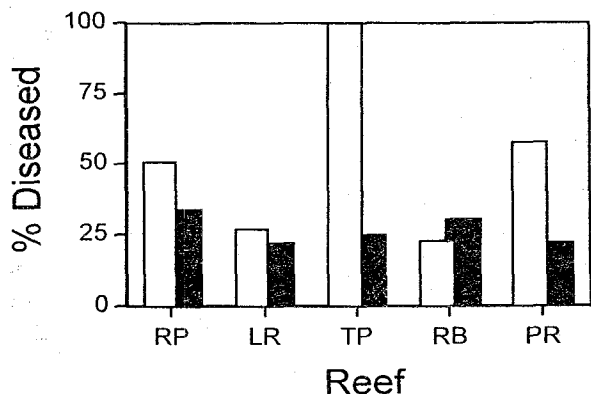


Fig. 6. Disease incidence in San Salvador, Bahamas, during a single survey in December 1996. Shaded bars represent *G. ventalina* and open bars *G. flabellum*. RP=Rocky Point, LR=Linsey Reef, TP=Telephone Pole, RB=Rice Bay, PR=Potters Reef.

but less virulent than the epizootic which occurred in the 1980s (Nagelkerken *et al.* 1997). Signs and progression of the disease, however, appear similar (Guzmán and Cortés 1984; Garzón-Ferreira and Zea 1992) and may have even been caused by a similar pathogen. In addition to the higher concentration of pigmented sclerites, sea fans can also produce anti-fungal compounds (Merkle *et al.* 1997). We are continuing to investigate the role of sclerites as a defense mechanism against pathogen invasion.

#### ACKNOWLEDGMENTS

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#### RESUMEN

Mortalidades masivas de abanicos de mar (*Gorgonia* spp.) en América Central y del Sur, se informaron en los 1980s. En algunos casos, las poblaciones nunca se recuperaron. Más recientemente se informa la mortandad extensa de tejido de colonias de *G. ventalina* y *G. flabellum* en todo el Caribe. Aunque la mortalidad de colonias fue menor en el evento más reciente, la distribución geográfica de la enfermedad parece ser mucho mayor. Muestras de tejido de colonias afectadas estaban infectadas por un hongo (*Aspergillus* sp.) el cual se demostró que causa la enfermedad en abanicos de mar sanos. Aquí, describimos observaciones sobre la fase temprana de infección y sobre la evolución posterior de la enfermedad. Basado en estas observaciones, informamos sobre la respuesta de *Gorgonia* spp. a la infección de *Aspergillus*.

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