Chesapeake Science Vol. 14, No. 4, p. 279–280 December, 1973

Short Papers and Notes

Studies on the "Lake Venice Disease" of Eurasian Milfoil in the Chesapeake Bay[1]

ABSTRACT: Eurasian water milfoil (*Myriophyllum spicatum* L.) which showed symptoms of "Lake Venice disease" was studied under laboratory conditions. In tanks containing distilled water, diseased plants produced only disease-free tissue. We were unable to produce symptoms of "Lake Venice disease" either by direct inoculation of healthy plants with extracts from diseased plants or by growing diseased and healthy plants in the same tank grown with Gro-Lux illumination. However, plants grown under low light intensity (indirect sunlight) and inoculated with extracts from diseased tissue produced symptoms resembling those of "Lake Venice disease." "Lake Venice disease" occurs on milfoil plants growing under stress conditions such as low light intensity, which increases the susceptibility of the plant to attack by microorganisms.

Introduction

In 1967, Elser described "various pathogenic conditions" of Eurasian milfoil (*Myriophyllum spicatum* L.) that resulted in a 99% decline in tonnage in the Chesapeake Bay. Elser recognized two sets of symptoms which he designated "Northeast Disease" (broken, stunted and stiff leaflets, flattened petioles fused into the basal leaflets), and "Lake Venice Condition" (flaccid stems and leaves covered with unusually heavy depositions of periphytic and epibiotic ooze).

Bayley, et al. (1968) studied the Northeast Disease of Eurasian milfoil and concluded that the disease was the result of a "virus, virus-like particle, or a toxin." They also found bacteria associated with the diseased plants, but they considered the bacteria to be secondary. Bayley, et al. (1968) did not study "Lake Venice disease," concentrating their attention on "Northeast disease." In more recent studies, Bayley (1970) was unable to identify causal agents for either "Northeast Disease" or "Lake Venice Disease"; milfoil plants inoculated with alfalfa mosaic virus, tobacco mosaic virus, tobacco ringspot virus, potato virus X or potato virus Y remained disease free.

During the summer of 1972, further studies on the decline of milfoil were made as part of the "Rhode River Estuary Interdisciplinary Research on a Watershed–Estuarine System of the Chesapeake Bay," sponsored by the National Science Foundation.

MATERIALS AND METHODS

Collections of healthy and "diseased" Eurasian milfoil were made periodically during the summer of 1972 from the Rhode River and its tributary, Muddy Creek. Milfoil plants were grown in 75.7-liter tanks containing distilled water at room temperature (ca. 24 C); light was supplied by a single 24-inch Gro-Lux fluorescent lamp.

To isolate possible bacterial or fungal systemic pathogens, plant tissue was surface-sterilized by immersing for 10 seconds in commercial chlorox (5.25% sodium hypochlorite) and rinsed in tap water. Unsterilized plant tissues were also ground in a mortar and pestle and streaked on potato dextrose agar or nutrient agar with a sterile inoculating needle to determine what microorganisms were present. Surface-sterilized, diseased milfoil was ground and the sap was filtered through coarse Whatman #40 filter paper to remove plant tissue. One ml of undiluted sap was injected into the stems of healthy milfoil plants using a sterile syringe.

Diseased and healthy milfoil were grown in the same tank to determine if the causal organism could be transferred between plants in water. Diseased milfoil was also surface-sterilized and grown in tanks to determine if new growth developed symptoms of "Lake Venice disease." To investigate the possible influence of light on disease symptoms, milfoil plants were grown in 75.7-liter tanks receiving only indirect sunlight and compared to inoculated and non-inoculated plants in a second tank which received continuous fluorescent illumination; plants were inoculated (using a sterile syringe) with a bacterial suspension isolated from surface-sterilized diseased plant tissue. Plants inoculated with distilled water were the control treatment. Free hand sections of naturally diseased leaves were made and examined microscopically for the presence of fungal mycelium or bacteria.

Results and Discussion

Similar to the early findings of Bayley, et al. (1968) in their investigation of the "Northeast disease" of milfoil, bacteria were frequently isolated from both surface and non-surface sterilized material. However, when bacteria were injected into healthy plant tissue growing under Gro-Lux illumination, no symptoms

[1]Supported by National Science Foundation project "Rhode River Estuary Interdisciplinary Research on a Watershed–Estuarine System of the Chesapeake Bay." Contract number G.I. 29906. Scientific Article number A1871. Contribution number 4782 of the Maryland Agricultural Experiment Station.

occurred other than necrosis at the point of inoculation. Inoculation of healthy plants with ground, diseased plant material likewise resulted in similar, limited areas of necrosis at the point of inoculation, indicating that if a pathogen were responsible for "Lake Venice disease," it could not be mechanically transmitted.

Healthy plants placed in the same tank with diseased plants remained healthy and new growth of diseased plants developed no new symptoms. Microscopic examination of diseased tissue showed intercellular necrosis limited only to the area of visible external symptoms; fungal mycelium was not detected in any of the sections.

Milfoil plants inoculated with a bacterial suspension and grown under low light intensity developed symptoms of "Lake Venice disease" within 2 weeks; the non-inoculated plants grew normally. Plants inoculated with a bacterial suspension and kept in continuous lighting developed no symptoms. We therefore conclude that the "Lake Venice disease" occurs after the plant has been predisposed by low light intensities and that the causal agent may be a bacterium, although the possibility that fungi or viruses may also be involved should not be overlooked. Further studies are needed to identify the bacteria isolated and to investigate the possible role of fungi and viruses in the development of "Lake Venice disease" in milfoil growing under varying light intensities.

LITERATURE CITED

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A Flowing Experimental System with Filtered and Temperature-Regulated Seawater[1]

ABSTRACT: A method of setting up an inexpensive and easily maintained system with flowing filtered and temperature-regulated seawater is described. Filtration is by a combination of filter bags and cartridges of various porosities that results in 1 μ-filtered seawater with particulate carbon concentrations of less than 30 μ-grams/liter. The temperature of the water is regulated by a titanium heat transfer panel. Water is then pumped to a head tank for distribution to experimental trays. Alarm systems for excessive temperature and water level fluctuations are described.

Laboratory investigations of the food chain dynamics of filter feeding invertebrates require a system that provides ample amounts of flowing temperature-regulated and filtered seawater. Many investigators have described modifications of seawater systems to insure seawater quality (Clark and Clark, 1964). However, experimental systems for the investigation of the energetics, e.g., feeding and biodeposition, of filter feeders must provide a sufficient quantity of water to satisfy the pumping requirements of these organisms, especially when adequate replication is needed to detect experimental differences. If interest is in the effect of the concentration and species composition of phytoplankton, the seawater must also be adequately filtered to remove naturally-occurring phytoplankton so that controlled amounts of known phytoplankton culture may be added to the filtered seawater. Standing culture techniques, in which the phytoplankton is added at periodic intervals, do not simulate conditions found in nature. Thus an experimental system with flowing seawater is a better method to investigate food chain dynamics. It is obviously helpful that such a system be inexpensive and simple to set-up and maintain. As part of a project investigating aquaculture food chains, we have over a period of three years developed such a seawater system that might be of interest to other workers. We have included a list of supplies for the special components of the system (Table 1) for convenience. The initial cost of the materials (without labor) is about $500. The disposable filter cartridges (about $1.50 per cartridge) are the only major operating cost.

A fiberglass tank with a capacity of 900 liters contains the heating and filtering apparatus (Fig. 1). The flow rate of the incoming raw seawater is controlled so that there is a small but continual overflow of unused water into the standpipe drain. The incoming seawater undergoes initial filtration by

[1]Contribution No. 3030 from the Woods Hole Oceanographic Institution. Supported by NSF Grant GI-32140. We wish to thank all the staff members of our project, especially J. Broda, S. Welch, and J. Davidson, who have contributed to the development of this system. We would also like to thank Drs. Carriker, J. Ryther and R. Backus who critically reviewed the manuscript.