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Fungi associated with decaying Spartina : final report

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Fungi Associated with Decaying Spartina

Final Report National Science Foundation Grant GA-31899

July, 1974

Frederick Y. Kazama

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Introduction

Although marine fungi have been studied since the mid 1800's, there have been relatively few physiological-ecological studies concerning the role of the fungi in the marine environment. Most studies have dealt with lignicolous or sediment-inhabiting fungi (see Johnson and Sparrow, 1961). After the importance of detritus in estuarine energy flow had been established (see Odum and de la Cruz, 1967 for references), there have been a number of papers dealing with the mycoflora of decomposing Spartina (see Gessner and Goos, 1973 a, b) and the mangrove (see Fell and Master, 1973; Newell, 1973). In these studies, lists of filamentous fungi and the number of times these fungi were isolated were presented and their possible roles were discussed. There have been no attempts, as far as I am aware, to study the physiological capacities of these isolates of saprophytic filamentous fungi. Thus, there have been a few synecological and still fewer autecological studies of the fungi involved in marine or estuarine detritus formation.

Gessner and Goos (1973 a, b) and Meyers, et al. (1970) have recently published papers describing the mycoflora of decomposing S. alterniflora. Their results seem to indicate differences between the mycoflora of decaying Spartina in New England (Gessner and Goos, 1973 a, b) and in Gulf Coast marshes (Meyers, et al., 1970). In New England,

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Sphaerulina pedicellata, Leptosphaeria typharum, Phoma sp., Septoria sp. and Alternaria sp. predominated while Fusarium, Phoma and Nigrospora predominated in Louisiana marshes. In both papers (and literature cited therein) it is obvious that a large number of the fungi associated with decaying Spartina are typically "terrestrial" fungi.

In the present communication the isolation of fungi associated with decaying Spartina in the York River, the Mattaponi River and on the Eastern Shore of Virginia is reported. Since the degree of tidal flushing and flooding differed considerably between the exposed Wachapreague Marsh (Eastern Shore of Virginia) (see Mendelssohn, 1973) and the lower salinity stations, it was decided that the litterbags would be placed in locations where they would remain completely submerged at all times. Under these conditions, the major difference. between stations was salinity. The Spartina in the litterbags served as bait. Therefore, in this study, the influence of salinity on the mycoflora was assessed. Hydrographic data collected during the sampling intervals are reported. Examination of the cellulolytic ability of the isolates and the influence of salinity on cellulolytic ability was also conducted.

Materials and Methods

Sampling and isolation procedures:

Healthy, green Spartina alterniflora and Spartina cynosuroides were harvested once in the spring for the June 1972 baiting. Dead, brown Spartina alterniflora was also harvested in late fall after the growing season (dje-back) for the November 1972 baiting. In the laboratory the leaf blades were removed from the sheaths. The "stems" and leaves were cut into 5 cm segments and oven dried at 90C for 12 hours. Approximately 10 g of the dried Spartina leaves or stems were weighed and then enclosed in aluminum foil. These packets were then autoclaved for 15 min. The autoclaved material served as bait.

Sampling (bait) containers were constructed from 16 oz polyethylene, wide mouth bottles with screw caps (Fisher Scientific Co.). Approximately 40 one centimeter holes were punched in each container and the bottle then lined with a nylon screen having a mesh size of $2mm^2$. These sample bottles were designed so that repeated sampling could easily be made without excessive handling of the sample containers. In the field, one packet of leaf material, one of stems, and a packet containing stems and leaves were carefully removed from the foil wrapper and placed in separate, surface sterilized containers and anchored at the various sampling sites. The bottles were anchored so that they were continuously submerged, but about 30 cm from the bottom. At approximately monthly intervals, pieces of material were

aseptically removed from the sample bottles, placed in sterile whirl-pak bags (Nasco), and kept on ice until they were brought back to the laboratory ·(2-4 hr after collection).

In the laboratory the material was removed from the bags and the free water blotted from the pieces. A #4 cork borer was used to remove discs of plant material whenever practical. These discs were then placed on various types of isolation media (see below). Three replicas of the various types of plates were used. Since the mean salinity of the various sampling stations differed, each media was prepared with either pondwater $(\mathbb{C}\%$ salinity, Stations H and M), seawater (30‰, Station W) or seawater diluted with pondwater (15‰, Station C). Initially (the first 5 samples), the following types of media were used for each sampling:

A. MV Medium

B. Rose Bengal Medium

Same as MV Medium except the antibiotics were left out and replaced by 0.67 g Rose bengal.

C. Spartina Medium

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D. Spartina - Rose Bengal Medium

Same as Spartina medium except the antibiotics were left out and replaced by 0.67 g Rose bengal.

E. Cellulose Medium

Acid swollen cellulose (Tansey, 1971) Soil extract (Aaronson, 1970) Vitamin mix* Streptomycin Penicillin G Agar Seawater, pondwater, or

appropriate seawater dilution 1,000 ml s.o g so .o ml 1.0 ml 0.5 q o.s g 15.0 g

* Thiamine, 2.0 mg; biotin, 0.01 mg; cyanocobalamin (B_{12}) , 0.01 mg; 100 ml distilled water

On the fourth and the eighth day after the original plating of the Spartina discs (later in the cycle the Spartina debris contained in the sample bottles was plated rather than discs) all the colonies (viewed at 15-20 X magnification through stereomicroscopes) growing out of the discs were picked by transferring a small multihyphal piece of agar to fresh plates containing the identical media, but without the antibiotics. If the colony on the second plate appeared pure, a small piece of mycelia was removed and tubed on MV slants. After adequate growth the slants were covered with sterile mineral oil and stored at 5 C. If the second isolated plate appeared contaminated, serial transfers were made until it was reasonably certain that pure cultures were present and these were then tubed and stored as above.

Fungi were also isolated by overlaying Spartina discs with the appropriate salinity water and scattering sterile, ground Spartina

on the water surface. After 7 days incubation period a sterile coverslip was touched to the water surface the adherent material transferred and spread on the surface of MV plates. Three days spreading, developing colonies were isolated. This procedure yielded mostly phycomycetes.

Cellulolytic activity (Viscometric method):

Preliminary experiments were conducted to determine the growth conditions necessary for the elaboration of cellulolytic enzymes (EC 3.2.1.4) (carboxymethylcellulase in particular). Since the number of isolates to be examined for enzyme activity was extremely large, the employment of crude assay techniques was unavoidable.

It was found that the following medium provided for the production of cellulolytic enzymes in a wide range of organisms. This medium was used exclusively in the survey since it seemed to allow good cellulolytic enzyme production in a wide range of organisms .

Some organisms that are capable of elaborating cellulases in the above medium were found to produce cellulases in other media . The medium described below was varied by using different carbohydrate sources.

Bacto-peptone CaCl₂ Yeast extract Trace metals (Kazama and Fuller, 1970) Tricine 1N KOH to pH 7.4 0.4 g 0.15g 0.1 g 1.0 ml 3.6 g NaCl is added in the following quantities O, 7.5, 12.0, 25.0 g/1 to simulate 0 , 10 , 15 , 32% salinity.
Distilled H.O to make 1.000 ml to make 1,000 ml As the carbonydrate source, 10 g/I of ground Spartina, 10 g/1 of Whatman cellulose powder, CF-11 (Ward R. Balston, Ltd., England), 10 g/1 of Alphacel (Nutritional Biochemicals) or 10 g/1 of carboxymethyl cellulose, Type 7 MF (Hercules Incorporated, Wilmington, Delaware) could be used.

The fungi to be tested were first grown on MV plates and discs cut from the edge of the colony with a $#4$ cork borer were then used as the inoculum. Erlenmyer flasks (25 ml) containing 10 ml of the growth medium and capped with aluminum foil were inoculated with a single agar disc containing the fungus. The cultures were grown for 7 days at 28 Con a shaker set at 125 RPM. After 7 days, the culture fluid was decanted into centrifuge tubes and centrifuged at 15,000 g for 10 min@ 5 c. The supernatant was then kept on ice and assayed for cellulase activity.

The reaction mixture consisted of 1.2% carboxymethyl cellulose (Type 7MF; D.P. about 1,100; D.S. 0.65-0.85; lot 99693, Hercules Incorp., Wilmington, Delaware) in 0.05 M citrate buffer at pH 5.6 . Cannon-Fenske viscometric tubes #300 were charged as recommended by the manufacturer (Cannon Instrument Co., State College, Pa.). The viscometric tubes were equilibrated to 30 c, the temperature at which the experiments were conducted. After equilibration, 1 ml of the culture fluid was added and examined for cellulase activity. Relative

activity (RA) was expressed as RA = 100 (t50)⁻¹ (ml culture fluid)⁻¹: tSO=time in min required for SO% reduction in viscoscity of the carboxymethyl cellulose solution. It was found that the introduction of various amounts of salts with the culture fluid did not materially influence the enzyme activity under the conditions used. Controls consisted of the culture fluids boiled for 15 min prior to the viscometric assays. Preliminary studies were conducted to determine pH values, substrate and enzyme concentrations, and salt requirements that would allow.for the detection and comparisons of cellulose activities in a large number of isolates. It was realized that conditions were not optimal for all isolates and the methods rather crude.

Cellulolytic activity (DNS method):

The fungi were grown as described in the preceeding section. Cellulolytic activity was determined essentially as described by Pettersson and Porath (1966). Reaction mixtures consisted of 2 ml of 0.6% carboxymethyl cellulose (Type 7MF, Hercules Inc., Wilmington, Del.) in 0.05 M citrate buffer at pH 5.6 and 0.2 ml of culture fluid. The reaction was allowed to proceed for 30 min at 30 C. The reaction was stopped by placing the tubes in boiling water for 10 min. Three ml of DNS reagentwereadded to the reaction mixture, heated in a boiling water bath for 10 min and after cooling, the absorption determined on a Spectronic 70 at a wave length of 640 nm. Blanks and controls consisted of the same reaction mixture except that the culture fluid was boiled for 15 min before being added to the reaction mixture.

A standard curve of reducing sugar equivalents was constructed utilizing glucose.

This method of determining enzyme activity was abandoned for the purposes of this survey when it was shown to be less sensitive than the viscometric method (Table XI) and more sensitive to presence of salts.

Cellulolytic activity (agar diffusion assay):

During the course of the study, it became obvious that the large number of fungal isolates from decomposing Spartina made the viscometric method of screening organisms for cellulase activity impractical. Therefore, the agar diffusion assay was to a large extent used to cope with the large number of isolates.

The agar diffusion technique was essentially that described by Tansey (1971), but with some modifications. Acid swollen cellulose was prepared as described by Tansey (1971). The swollen cellulose-agar media consisted of:

Stir continuously and dispense 8 ml/test tube (16 x 150 mm). Autoclave 20 min. When the tubes cooled, but prior to the solidification of the media, the tubes were placed on an omni-mixer and the cellulose resuspended. After the contents were thoroughly mixed, the tubes were immediately placed at 5 C to rapidly set the agar.

Each isolate was examined for the ability to clear acid swollen cellulose at 30% (seawater), 15% (seawater: pondwater, 1:1), and at 0% (Haynes Mill pondwater). The inoculated tubes were incubated \Box at 27 C and the depth of clearing measured after 30 days.

Sampling stations:

The hydrographic characteristics of the sampling stations are described in the next section and the stations are referred to as Stations H, M, C and w. In the results, Station H has been divided into Stations HA and HC. HA refers to results obtained using Spartina alterniflora as bait, while HC refers to results obtained with Spartina cynosuroides. Sampling HC was discontinued when the large number of fungal isolates obtained made it impractical to continue further. All discussions are based on results obtained from Station HA. Also, in a.few tables and graphs Station F and sampling MN are included. Data was being collected at Station F, a fresh water site, until the samples were vandalized. Collection MN consisted of isolates from drift Spartina. Both the results of Station F and sampling MN, like Station HC, have been presented for the sake of completion, but have not been discussed.

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. Bampling Sites and Hydrographic Data

Figure 1 shows the location of the four sampling sites, while Figs. 2-5 summarize the hydrographic data collected at each sampling period. Temperature was determined by placing a thermometer in the water column over the sample bottle. The pH of the overlying water was determined using a Beckman Zeromatic SS-3 pH meter. The water was brought back to the laboratory in 50 ml medicine bottles which were kept on ice in an ice chest. Salinity was measured using a Beckman Model RS7B salinometer. Dissolved oxygen was determined by the modified Winkler method (Strickland and Parsons, 1968).

Results

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Although attempts were made to quantitate litterbag loss of Spartina (see Other Studies section below), technical difficulties prevented an accurate assessment of the process. However, visual observation of the plant material showed that decomposition occurred more rapidly at the lower salinity stations (Hand M) than at the higher (C and W) salinity stations. Spartina harvested after the growing season (die-back) and set out in the late fall showed that at the lower salinity station (Station H) Spartina leaves set out in November 1972 remained nearly intact until March 1973 and then were rapidly decomposed. Stems set out at the same time remained largely intact until March 1973, but were well macerated by May 1973. Identifiable stem fragments (well macerated) were still present in July 1973 when observations were discontinued. At the highest salinity station (Station W), identifiable, but well macerated leaves and relatively intact stems were.present in July 1973 when observations were discontinued. Plant material (spring growth) set out in June 1972 showed the same phenomenon - more rapid decomposition at the lower than at the higher salinities. Decomposition was nearly complete (except for some fiberous material) by November 1972. Apparently material set out in the spring decomposes more rapidly than material set out in the fall (Kirby, 1971). During the fall (Sept. - Oct.),

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sample containers at Stations C and W experienced very heavy fouling by oyster spats, barnacles, tunicates, crabs and other invertebrates. Fouling at the lower salinity stations (Hand M) was mostly due to algal growth. The abundant animals within the sample containers (entered as larvae) probably contributed to the loss of material from the sample containers •. All sample bags experienced periodic siltation; however, the presence of the above invertebrates probably indicates that anaerobiosis did not exist for long periods.

Table 1 lists the taxa of fungi isolated into pure culture from decomposing Spartina at each station. The list also describes the number of isolates of each taxa that were obtained from each station at each sample period. The total number of isolates of each taxa has been tabulated. In Table II the same information as in Table I has been arranged so that the fungal population between stations can be compared.

The genera Trichoderma, Penicillium, Gliocladium, Acremonium, Fusarium and relatively large number of phycomycetes were present at all stations (Table II). The phycomycetes could not be identified further since the mode of zoospore discharge and the type of sex organ must be known before positive identification can be made. The amount of time required for the manipulations and observations prevented further identification of these "sterile", non-septate mycelia.

In general, it appears that more taxa are found at the lower salinity stations (H, M) compared to the higher salinity stations. The data indicates that the number of isolates of some genera like

Trichoderma and Fusarium decrease with increasing salinities (Table II), while the numbers of Penicillium and Flagellospora increase with increasing salinities. The distribution of other genera, such as Acremonium seems to be independent of salinity. Some organisms occurred at all stations while others were restricted to the lower or higher salinity stations. Similar types of differences in distribution can be found among the various species within a genus. Many fungi were only infrequently recorded. There are differences in the time period in which certain fungi are isolated - some early, others late in the degradation cycle.

Several interesting seasonal features arose when the "winter" (below 10 C) and "summer" (above 10 C) mycoflora were compared. Figs. 6-10 show that the filamentous phycomycetes were frequently isolated only during the winter at stations C and W, while at the. lower salinity stations there appears to be a minor peak approximately in August in addition to the winter peak. Comparisons between the number of fungi and taxa isolated at the various stations during summer and winter show that a greater diversity occurred during the summer than the winter (Table III). More fungi (isolates) are obtained during the summer than in the winter; however, the ratio of isolates/ taxa was found to be higher in the winter than in the summer (Table III).

No relationship was seen between pH and the fungal populations.

Although many of the fungi isolated from decomposing Spartina have previously been reported from the marine environment, no reports have been published on the possible roles (physiological potential) of these fungi. In this regard, we examined all the isolates for their

ability to participate in cellulose decomposition (cellulolytic activity). The agar diffusion assay was primarily utilized. Because a large number was dealt with, the employment of crude methodology was unavoidable. Since the Spartina fragments can easily be transported withinan estuary or encounter a range of salinities within marshes (depending on precipitation, tidal heights, evaporation, etc.), we assayed for cellulolytic ability of each isolate at o, 15, *30%o* salinity, irrespective of the mean salinity of the station at which the organism was isolated.

Appendix I lists the isolates obtained at each station and the depth to which cellulose was cleared. This appendix was included to show that individual isolates must be considered when cellulolytic ability is being examined. For example, isolates of Penicillium notatum show a range of cellulolytic abilities at the various salinities. Some are able to clear acid swollen cellulose at all salinities, others only at the two lower salinities, and a few only at the highest salinity. For individual isolates, the range of salinities which was recorded at each sampling station was no indication of its potential to clear cellulose at salinities outside of the range. Finally, it should be obvious that the following discussion on the behavior of the fungi at the various salinities is based on averaging the response of either a particular species or taxa and may not hold for any particular isolate.

Tables IV and V summarize the data found in Appendix I. There do not appear to be any seasonal trends in the number of cellulose

decomposers at each station (Table III). Although differences in the number of cellulolytic fungi are found when different stations)~*,;~ are examined at any given sample period, these differences are probably artifacts due to the relatively small sample size examined at each station at any one sampling period. The data show that the proportion of fungi at all stations capable of clearing swollen cellulose is lower at the higher salinities than at the lower (Table IV).

Comparing the proportions of fungi capable of clearing cellulose at 0, 15, and 30% at the stations with different mean salinities provided interesting trends (Table V). At all stations, the percent isolates capable of clearing cellulose at 0% was approximately the same. However, a greater proportion of the fungi isolated at the higher salinity stations (C, W) was capable of clearing cellulose at 15% than was found for the lower salinity stations (H, M) . A greater proportion of the population isolated at the highest salinity station (Station W) was able to clear cellulose at 30% than was found for any other station, with Station C showing an intermediate value between Station W and the two lower salinity stations (H, M) . Within each station the disparity between those able to clear cellulose at 0 and 30% decreases with increasing mean salinity of the stations. Thus, as the salinity of the station increases, the proportion of the population capable of cellulose clearing at higher salinities increases.

Tabulation of the number of genera, rather than total taxe $\mathcal{R} \rightarrow$ capable of clearing cellulose gave results comparable to those described above for the tabulated isolates (Table VI). There appears

to be a larger number of genera capable of decomposing cellulose at the lower salinities than at the higher . A large percentage of the $,$ genera had members which were capable of clearing cellulose. There were fewer genera capable of clearing cellulose at 30% than at 0% salinity, the value being intermediate at 15‰. At the highest salinity station (W) a greater percentage of the genera were capable of clearing cellulose at 30% than at the stations H, M, and C. Comparison of data from Table IV and Table VI show that the ratio of isolates capable of clearing cellulose to the number of genera capable of clearing cellulose is 6.1 at Station H, 8.9 at Station M, 9.6 at Station C and 11.l at Station W. Thus, it appears that larger numbers of fewer genera of cellulose decomposers were present at the higher salinities than at the lower.

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Discussion

It is well known that any method used for fungal sampling is selective to some extent and may limit the array of species recovered. (Barut and Johnson, 1962; Park, 1972). Consequently, no single method of isolation is expected to provide the full complement of species. Although the number of isolates obtained from decomposing Spartina sp. has been used and treated as meaningful numbers in the discussion below, it must be strongly emphasized that standardization in the amount of inOculum was not possible. Initially discs of Spartina were easily obtained; however, after decomposition had progressed and the tissues became water-logged and sodden, it became increasingly difficult to get standardized amounts of tissue. Also, during the isolation procedure, all colonies which emerged from the plant material were isolated. Whether each colony represents a single ramifying colony within the decaying tissue or discrete reproductive units is not known. Furthermore, fungi which were capable of rapid vegetative growth on the isolation medium were probably favored over the slower growing fungi. As the isolation of the fungi required some laboratory manipulations before enumeration, the failure to obtain a fungus is also not evidence that it is not present.

In spite of these difficulties, it is felt that the numbers obtained can be used comparatively. Since a standard procedure was

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utilized, it is felt that the differences are probably real, but that the significance of the differences is somewhat obscure.

General considerations show that the list of deuteromvectous *··,t.* genera associated with decomposing Spartina appears to bro~dly resemble those reported for estuarine salt marsh and marine sediments (Barut and Johnson, 1962; Siepman, 1959 a, b; Elliott, 1930), mangrove leaves (Fell and Masters, 1973) and seedlings (Newell, 1973), and to a large extent, resembles terrestrial soil mycoflora (see Wicklow, 1973, and literature cited therein). The mycoflora reported here differs substantially from that reported for wood inhabiting fungi of the marine environment (Jones, 1968; Shearer, 1972). Gessner and Goos (1973 a, b) found that the fungal flora of decomposing Spartina differed depending on whether direct microscopic observations or dilution plating was used. Direct microscopic examination showed. marine ascomycetes and deuteromycetes, while pour plates yielded primarily terrestrial deuteromycetes and also terrestrial ascomycetes and several marine deuteremycetes. The mycoflora observed by the pour plate method resembled those fungi obtained in the present study. Meyers, et al. (1970) and Day, et al. (1973) suggested that beside the yeasts, the genera Fusarium, Phoma and Nigrospora were the most abundant organisms found in association with Spartina.

None of the studies reported the occurrence of abundant phycomycetes during certain periods as was found in decomposing Spartina during \cdot . the winter in Virginia. Although not included in the results of this report, monocentric fungi with biflagellate zoospores (probably

Thraustochytriaceae) could invariably be isolated from decaying Spartina at Stations C and W anytime during the year, and at times at Station M. They were almost never isolated $a \in \mathbb{S}$ Station H. (Pollen or dried ground Spartina was used as bait and was scattered over the surface of the water containing bits of Spartina from the litterbags.)

There are several possible reasons for the difference in mycoflora observed on decomposing Spartina by the various authors. Perhaps some of the differences in the saprophytic mycoflora of different substrates in the marine environment may also be attributed to these reasons. It is possible that differences in location (New England, Virginia, Louisiana) (or substrate) are responsible for the differences in mycoflora. Yet considerations put forth by Stanier (1953) would suggest that saprobic microorganisms respond to microenvironments and any particular type of microenvironment is as likely to occur in one geographical area as in another. This argument could be used to explain the cosmopolitan distribution of a large number of saprophytic microorganisms (Pirozynski, 1968). Compare for their similarities, for example, the fungal genera and species composition of the mycoflora of manipulated praire stands (Wicklow, 1973), of mesic conifer-hardwood forests (Christensen, et al., 1962; Christensen, 1969), of salt marshes (Elliott, 1930), of leaves of Halimione portulacoides (Dickinson, 1965), and of organic detritus in streams (Park, 1972). It seems then that the differences in isolation techniques could be mainly responsible for the difference observed in mycoflora of decomposing Spartina.

Direct observation of the substrate and subsequent picking of spores from fruiting structures would tend to emphasize those organisms that are able to fruit under the isolation conditions and those with highly visible fruiting structures or spores. Likewise, under these conditions "sterile" mycelium, phycomycetous fungi, and those fungi with inconspicuous or small spores would be underrepresented.

Therefore, until standard techniques and much more effort is employed, it will not be possible to assess the specific fungal population responsible for the decomposition of Spartina and their possible ecological roles. That the studies of the fungal populations reported to be responsible for Spartina degradation should possibly show greater similarities is based on the recent report by Park (1972) that suggests that the mycoflora of freshwater detritus bears a relationship to the organic material substrate rather than to "aquatic" or "terrestrial" designations and the reports mentioned above which indicate a large degree of similarity among the saprophytic flora of decaying organic substances.

Beside the overall similarities of the mycoflora of decaying Spartina and that of decaying plant material and marine sediments, the present study showed some other interesting distributional patterns. Borut and Johnson (1962) in their survey of fungi in estuarine sediments reported that none of the species exhibited a definite hydrographic or seasonal distribution pattern. Gessner and Goos (1973 a, b) reported that "fungi most frequently identified were found with equal abundance along the salinity gradient." Honnk (1957) reported changes

in the mycoflora of sediments with changes in salinity. His data has been summarized by Johnson and Sparrow (1961).

Common soil fungi such as Aspergillus, Penicillium, Cephalosporium, Trichoderma, Alternaria and Cladosporium appear to be ubiquitous and have been frequently recorded in estuarine sediments, water, sand of tide-washed dunes, and on wood submerged in the marine environment (see Johnson and Sparrow, l961 and Borut and Johnson, 1962 for references). Johnson (l968) reports that these organisms "occur with remarkable frequency in marine and estuarine sediments." However, differences in the distribution of these organisms along a salinity gradient have not been recorded. LHOnnk (1957) presented some distributional data on the fungi of sediments, but his major emphasis was on the phycomycetes and the paucity data on the Ascomycetes and Fungi Imperfecti makes the information difficult to interpret.] Even with the severe restriction that the numbers of fungi obtained in this study can only be used comparatively, there seems to be considerable evidence that most of the genera of "terrestrial" fungi are isolated with decreasing frequency with increasing salinities while a few seem to be increasingly isolated with increasing salinities and still others appear to be isolated independent of salinity. (Although a wide range of salinity is encountered at each station, there is a a distinct difference in the mean salinity of Stations Hand M, C and W). For example, the differences in the number of isolates of Trichoderma and Penicillium seems to be related to differences in the salinities of the stations. A larger number of different genera (diversity?) were found at the lower salinities than at the higher salinities indicating furthermore that salinity dependent distributions occur.

Criticism can be raised that the above results are due to the isolation conditions which are highly selective and that the abundance and rapidity of growth of some isolates prevented the isolation of other fungi. Nevertheless, this does not exclude the possibility that these organisms may be the ones which can compete successfully or can colonize the substrate at a greater rate or to a greater extent.

Fluctuations in the seasonal occurrence of saprophytic filamentous fungi in the marine environment have been infrequently recorded (Seipman, 1959 a, b; Elliott, 1930). Elliott (1930) reported that of 6 different sampling months, June was the most favorable. The seasonal occurrence of the phycomycetes, as reported here has not been previously recorded for the marine environment, although evidence of similar types of seasonal fluctuations abound in the literature dealing with the ecology of freshwater Phycomycetes (Sparrow, 1968 for references).

Again, the question of inadequate sampling techniques could be raised on the occurrence of the seasonal patterns. However, the use of the same sampling procedure throughout, would indicate that the seasonal differences are real. Whether other more specific seasonal patterns occur is difficult to ascertain. The rather large difference between the number of fungal taxa found below and above 10 C would indicate that there are other seasonal differences. For example, at Stations H, M, and C Trichoderma harzianum appears to be absent during the "winter" months (samples 7-10) and present during other periods of the year. However, as mentioned earlier, failure to isolate a fungus does not mean its absence prevents a vigorous interpretation of the data. Furthermore, during the course of the study

the substrate was probably continuously changing and to determine whether a specific cyclic annual pattern occurred would require a different type of sampling program.

No easily identifiable successional pattern was observed. Certainly, different groups of fungi appeared only after certain intervals; however, the limitations on methodology prevents confident use of the data. Newell (1973) reported what he believed was a successional pattern during the decomposition of mangrove seedlings during a five month period. He found a decrease in the hyphomycetes along with a rise in the number of ascomycetes and Sphaeropsidales. Several factors tended to make successional patterns difficult to ascertain in this study and similar studies of decomposition in the estuarine situation utilizing particle plating techniques. For example: (l) Does the inability to isolate many ascomycetes early in the sequence mean that they are absent, or are they initially masked by the rapidly growing hyphomycetes? (2) Do we know enough about the physiological capacities of "marine" fungi to assign possible ecological roles to these organisms? For example, we need to know the influence of salinity on substrate uptake and elaboration of enzymes of the "terrestrial" marine flora. (3) How does periodic flooding on input of organic and other substances (Odum, 1971; Squiers and Good, 1974) influence the fungal population? (4) What is the contribution of the fungi from terrestrial run-off and the air spora to the population and activities of the "marine" fungal population? In soil, Burges (1958) estimated that fungal hyphae have a life of 1-3 days at the most. Thus, it seems that a knowledge of the life span of fungal

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mycelia, influence of fluctuating physical and chemical parameters, understanding of the physiological potentials of the "marine" mycoflora as well. as the potential immigrants, knowledge as to whether the substrate merely acts as an attachment surface, and sampling at frequent intervals using several different techniques are required before a pattern of marine fungal succession on a particular substrate can be deliniated with confidence. Importantly, the ecological role _of fungi cannot. be assumed on the basis of frequent isolation or detection in particular habitats, but that the role or significance must be determined by investigation (Park, 1972) and established in situ.

Among the studies dealing with the ecology of filamentous marine fungal saprobes, very few, with the exception of salinity tolerances, have dealt with the physiology of these organisms as related to tneir possible roles in the marine environment. Nearly all other previous physiological studies of marine fungi have dealt with the lignicolous fungi or the "true" marine fungi which are only found in the marine environment.

The information on the ability of the isolates to clear cellulose at the various salinities presented in this study pointed to some phenomena which may have been previously expressed, but not shown for marine fungi. It was found that the percentages of isolates (or genera) at each station capable of clearing swollen cellulose (at any salinity) was approximately the same, but with increasing mean salinities, the proportion of fungi capable of clearing cellulose at 15 and 30% increased. The difference between those able to decompose cellulose

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at O and 30k decreased with increasing salinities. The number of different genera represented also was shown to decrease with increases in salinity of the stations.

The above data can be interpreted in several ways. First, there is a strong suggestion here that terrestrial and freshwater.imputs may be an important source of the fungi participating in Spartina decomposition, especially at the lower salinity locations of estuaries. Gessner and Goos (1973 a, b) and Meyers, et al. (1970) had previously reported abundant fungi on both live and "dried" (after die-back) Spartina prior to deposition on the marsh surface. Secondly, the data could suggest that there is a process occurring whereby certain "terrestrial" fungi with physiological attributes enabling them to compete successfully in the marine environment are selected. The evidence indicates that fungi which are capable of increased salinity tolerances maybe those which are selected (see, for example, Trichoderma harzianum). Johnson (1968) in summarizing previous studies on common \sim soil inhabiting fungi in the marine, including estuarine, environment stated "there is little doubt that representatives of such genera as Alternaria, Cladosporium, Fusarium, Trichoderma, Gliocladium, Gymnoascus, Aspergillus and Penicillium occur with remarkable frequency in marine and estuarine sediments." Our data on some of these organisms found on decaying Spartina suggests that salinity influences their distribution· and that they are not universally abundant nor do they exist with unchanging frequency as implied by the statement of Johnson (1968). Again the reader is cautioned of the limitations of the sampling methods in relation to the use of the terms "abundance" and "frequency".

Thirdly, the data can be interpreted to mean that there is a population of fungi, including "typically" terrestrial fungi, that are physiologically adapt. to highly saline conditions.

Very few previous studies of saprophytic fungi associated with decomposition of plant litter in the marine environment have dealt with physiology. Most studies (see Gessner and Goos, 1973 a, b for references) assume that presence of a particular fungus implied some kind of degradative activity. Our data suggest that although many fungi are capable of growth at relatively high salinities (15-32%) their ability to participate in a physiological activity (for example cellulose decomposition) may differ at different salinities. In addition, there may be strain differences with regards to physiological responses to salinity.

Borut and Johnson (1962) examined gelatin and pectin liquifaction and starch and cellulose degradation of 20 isolates of sediment inhabiting fungi of the Neuse-Newport system in North Carolina. They examined cellulose decomposition in a mineral medium with only a trace of Nac1·and recorded 65% being able to decompose cellulose to varying degrees. No data was presented on the influence of salinity on decomposition. Our data showing that approximately 65% of the total number of isolates are capable of decomposing cellulose compares favorably to that found by Borut and Johnson (1962) on sediment inhabiting marine fungi and by Park (1972) on freshwater, detritus associated fungi. Also of interest in the study of Borut and Johnson (1962) was that some fungi could grow well in salinity-temperature

stresses, but under the same stresses, germinated poorly or not at all. Perhaps this, in addition to our evidence of decreased physiological activity of many "soil fungi" with increasing salinities, indicates the importance of constant terrestrial imputs of these fungi rather than a self-maintaining autochthonous population.

Sui (1951) compiled extensive data for a large number of fungi capable of decomposing cellulose in the terrestrial environment. On the whole he found that the population of fungi associated with decay of cotton fabric resembled that of soil. The same relationship seems to exist for the cellulose decomposers of Spartina where the mycoflora resembles the mycoflora of the marine sediments. Although comparisons are difficult because of the diversity of organisms, in general, the same organisms responsible for the decomposition of cotton fabric in the terrestrial environment seem to be capable of participating in the decomposition of cellulose of Spartina in the marine environment. This seems to be true at least at the low : salinities (compare data presented in this study and Sui, 1951, Chapter 4).

Finally, the data on the fungal flora and cellulose decomposition seem to fit the pattern observed in the loss of material from litterbags. Our field observations showed that decomposition occurred more rapidly at lower salinities and during summers. Kirby (1971) reported more . rapid litterbag losses during spring and summer than during winter and K. Moore (personal communication) found that particulate organic carbon and dissolved organic carbon, which can be used as an indicator of decompositional activity, initially rose more rapidly in the spring in the lower salinity marshes than in the higher. The greater number

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and possibly activity of fungal cellulose decomposers at the lower salinities and during the warmer months seem to correlate with the above observations. They are probably contributing factors in the decomposition of marsh plants.

Other Studies:

Examination of litterbag loss.

During the early phases of the study, we initiated a study attempting to correlate litterbag loss (weight loss) with the cellulose decomposing mycoflora. Both Whatman filters and *oven* dried Spartina were incorporated into litterbags of 25 sq inches and having a mesh size of 500 μ m. Although a small sample was utilized for the initial experiment, considerable difficulty was encountered in the treatment of the samples (unreported data):

- a). After approximately one month, most bags showed dry weight gains. This was due to sedimentation and fouling.
- b). Since sedimentation and fouling seemed to be a problem we attempted to wash the contents of the bags using running tap water or by agitating the bags in water. During these manipulations the soft waterlogged pieces of Spartina, especially leaf material, and the Whatman paper fragmented and·were lost through the meshes.

These initial experiments indicated that an accurate and easy method of determining litterbag loss of submerged material is difficult to achieve. Therefore, no further studies were undertaken. Gessner and Goos (1973 a, b) encountered similar difficulties. However, they reported a SO% loss of dry weight in the grass exposed on the marsh for 6 months, while de la Cruz (1965) reported a 50% loss of material from litterbags submerged in a creek for 3 months.

Kirby (1971) indicated that the loss from litterbags was a function of the mesh size, area in which the bags were placed, the amount of flushing received,and the temperature. He reported, as did de la Cruz (1965), a more rapid loss of material from bags placed in a tidal channel than from bags placed in high marsh areas.

A recent study (Anderson, 1973) on the leaching and decomposition of leaflitter of deciduous and woodland soils indicated most of the weight loss during the first 20 months was due to leaching rather than microbial decomposition. This indicates that the abiotic breakdown processes occurring during Spartina decomposition must be known before the weight losses from litterbags can be properly evaluated.

In situ production of cellulolytic enzymes.

Most (greatest number isolated) of the fungi that were isolated and identified from the initial samples were deuteromycetes (Fungi Imperfecti). Since there has been some question as to whether these "terrestrial contaminants" are active in the marine habitat, it was desirable to determine whether cellulases could be elaborated by these organisms under in situ conditions. Four isolates, which were found to be active producers of carboxymethylcellulase (determined by the viscometric method), were allowed to grow on ground Spartina alterniflora at 0 and 30% for 5 days on a shaker. The mycelial balls with the adherent ground Spartina were then rinsed with either sterile pondwater (0% salinity) or .seawater (30%). The washed mycelia (with the adherent Spartina) were then placed in prewetted and sterile dialysis tubing. Approximately 3 ml of the appropriate water was then added to the dialysis tubing. Carboxymethylcellulase activity was determined and the tubing knotted to prevent contamination. The tubes were then transported to Station M and anchored in situ.

After 1 week the tubes were retrieved. The tubing was surface sterilized, cut open, and the cellulase activity of the liquid determined. Spread plates of 0.1 ml aliquots showed that no contamination had occurred during the in situ exposure. The results are presented in Table VII. The data indicates that these typical terrestrial isolates were capable of elaborating cellulolytic enzymes under in situ environmental conditions. Thus, given the opportunity, it is very likely that "terrestrial" fungi are able to contribute to the decomposition of Spartina in estuarine marshes.

Isolation on various types of media.

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During the initial 3 sampling periods, 5 different isolation media were tested to determine which would allow the isolation of the greatest diversity of fungi. The results are given in Table VIII. The MV medium was shown to yield the greatest diversity. It was obvious that several different media should be utilized. However, the large number of isolates found on all media made it impractical to use more than a single medium for this study. Since MV yielded the greatest diversity, it was selected as the primary isolation medium.

Effects of nitrogen and phosphorus on the enzyme activity of two isolates of Trichoderma koningii.

· During the initial phases of our studies we found that growth of many isolates of Trichoderma sp. on only ground Spartina of approximately 200-250 μ m (passed through 250 μ m, but retained on 200 μ m mesh screens) was poor and low or no activity of cellulases was detectable by viscometry. After several preliminary experiments we found that a medium containing 10 g of ground Spartina, 0.4 g of urea, Bacto-peptone, $KH_{2}PO_{A}$, (NH₄)₂SO₄ and 1 liter of water of the appropriate salinity allowed good growth, as determined visually, and allowed the elaboration of carboxymethylcellulase by many isolates of Trichoderma sp. It was of interest to determine which of the added chemicals allowed for the increased cellulase production observed. Table IX shows the effects of the addition of single chemicals to a basal medium of 1% ground Spartina in seawater or pondwater.

The results showed that nitrogen in the form of $(\text{NH}_4)_2$ SO4, but not KNO_z, allowed the elaboration of large quantities of enzymes which were undetectable in the controls. The addition of urea stimulated cellulase production in one of the isolates grown at 30%. The addition of nitrogen in the form of Bacto-peptone also gave variable results with one of the isolates being stimulated to produce cellulolytic enzymes. Addition of phosphate as KH_2PO_4 stimulated cellulose elaboration, but to a lesser extent than did ammonia.

Various C:N ratios have been reported for dead Spartina alterniflora (Squiers and Good, 1974; Thayer, 1974) and these generally
have been much higher than the 25-55:1 ratio of carbon to nitrogen utilization recorded for fungi during the utilization of cellulose (Siu, 1951). Although extremely speculative because of the paucity of data presented here, there is a possibility that the rate of decomposition of Spartina may be governed to some extent by the availability of nitrogen. Cellulolytic enzyme production appears to be sensitive to additions of nitrogen and would influence rates of cellulose decomposition. Kirby (1971) found that nitrogen was a limiting factor in his laboratory studies of Spartina decomposition. Comparisons between viscometric and agar diffusion assays.

Viscometric analysis of cellulose activity, following the changes in the degree of polymerization, is considered to be rapid and.precise. During the initial stages of hydrolysis, it is considered to be more sensitive when compared to procedures based on an increase in reducing end-groups, change in turbidty of cellulose suspensions or weight loss of material (Scharpe, et al., 1973; see Halliwell, 1963). Rautela and Cowling (1966) found that the rate of clearing of acid swollen cellulose was at least as well correlated with breakdown of "native" cellulose compared to weight loss, tensile strength loss and productivity of reducing sugars from carboxymethylcellulose.

Results obtained in the present study (Table X) showed that there was a poor correlation between carboxymethylcellulase activity and clearing of swollen cellulose. Reese (see "Discussion" in Halliwell, 1963) found poor correlation between carboxymethylcellulase activity and cellulolytic activity measured with undried cotton fibers as substrate. Since the meaning of the results presented in Table X were unclear and the procedure for the preparation of viscometric analysis time consuming, viscometry was abandoned. Studies utilizing the clearing of acid swollen cellulose as means of detecting cellulolytic activity are numerous (see Tansey, 1971).

37

Several interesting problems pertaining to the general ecology of marshes arose during the course of this study and should prove to be profitable if pursued.

- (1) Since microorganisms are thought to contribute to the food value of detrital particles (Odum and de la Cruz, 1967; Barlocher and Kendrick, 1973) the question arises as to whether the food quality of detritus varies with differences in salinity. The differences in microbial flora would suggest that differences in quality may be found.
- (2) Since the mycoflora was found to be mixed and differences in the ratio between phycomycetes (generally with cellulose walls) and deuteromycetes (with chitinous walls) varied, assessment of their biomass by averaging methods, such as the determination of total chitin, will prove to be difficult.
- (3) A comparison of the physiological parameters governing growth, especially temperature, between the marine phy comycetes and detteromycetes would be highly informative. Perhaps phycomycetes are less affected by low temperature at seawater salinities than are the deuteromycetes. Evidence of the existence of the "Phoma pattern" in .some deuteromycetes (see Johnson, 1968) and its absence so far among the phycomycetes studied indicates that the temperaturesalinity relationship differs between the two groups.
- (4) Some of the terrestrial counterparts of the fungi which were frequently isolated from decaying Spartina are known to produce antibiotics, toxins and other biologically highly active compounds Le.g. Penicillium, Fusarium, Gliocladium, Acremonium (Cephalosporium)J. It is interesting to note that species of Gliocladium produce gliotoxin, a substance which inhibits the growth ofPythium. Perhaps this kind of relationship, rather than temperature, directly affects the phycomycete/deuteromycete ratios which were found to vary seasonally. The general phenomenon of the interaction and competition of microorganism through allelochemic substances (Odum, 1971) in the marine environment is largely unexplored.
- (5) In Virginia, primary productivity is greater in freshwater and low salinity marshes than in the high salinity marshes of the Eastern Shore (Mendelssohn, 1973). Data by Mendelssohn suggested that the dead standing crop is a potential source of nutrients and in the marshes may be significant towards supporting their own growth. Perhaps the larger numbers and diversity of cellulose decomposers at the lower salinities contribute to a more rapid turnover of substances which contributes to the greater productivity found in the lower salinity of freshwater marshes. Observations on the relationships between litterbag losses, nutrient turnover, microbial flora, productivity in relation to salinity should prove to be illuminating.

39

Summary

- 1. There appears to be a seasonal pattern in the mycoflora associated with decomposing Spartina retained in completely submerged litterbags. Filmentous phycomycetes are abundant mainly during the winter (water temperature below 10°C).
- 2. Approximately 65% of the fungi isolated by particle plating are capable of clearing acid swollen cellulose regardless of the mean salinity of the station. However, with increasing mean salinities of the sample stations, increasingly greater proportions of the fungal population are capable of clearing cellulose at the higher salinities (15 and 30%).
- 3. More fungi (isolates and diversity) and greater numbers able to decompose cellulose seem to be found at the lower salinity stations than at the higher salinity stations.
- 4. Individual isolates and the physiological capacity of individual isolates at different salinities must be examined before the ecological role of a fungus can be established. There appear to be strain differences and it is difficult to generalize about physiological activities of various genera and species.
- 5. In many instances, there seems to be a salinity dependent distribution of various groups of fungi.
- 6. Typical "soil" fungi seem to be prevalent on decaying Spartina; however, individual ability to clear cellulose at different salinities varies.

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LITERATURE CITED

- Aaronson, S. 1970. Experimental microbial ecology. Academic Press, New York. 236 p.
- Anderson, J.M. 1973. The breakdown and decomposition of sweet chestnut (Castanea sativa Mill.) and beech (Fagus sylvatica L.) Leaf litter in two deciduous woodland soils. I. Breakdown, leaching and decomposition. Oecologia (Berl.) 12:251-274.
- Barlocher, F., and B. Kendrick. 1973. Fungi in the diet of Gammarus pseudolimnaeus (Amphipoda). Oikos 24:295-300.
- Borut, S.Y., and T.W. Johnson, Jr. 1962. Some biological observations on fungi in estuarine sediments. Mycologia 54:181-193.
- Burges, N.A. 1958. Micro-organisms in the soil. Hutchinson, London. 188 p.
- · Christensen, M.. 1969. Soil microfungi of dry to mesic conifer-hardwood forests in northern Wisconsin. Ecology 50:9-27.
- Christensen, M., W.F. Whittingham ang R.O. Novae. 1962. The soil microfungi of wet-mesic forests in southern Wisconsin. Mycologia 54:374-388.
- Day, J.W., Jr., W.G. Smith, P.R. Wagner, and W.C. Stowe. 1973. Community structure and carbon budget of a salt marsh and shallow bay estuarine system in Louisiana. Pub. No. LSU-SG-72-04. Center for Wetlands Resources, Louisiana State Univ., Baton Rouge.
- Dickinson, C.H. 1965. The mycoflora associated with Halimone portulacoides. Trans. Brit. Mycol. Soc. 48:603-610.
- Elliott, J.S.B. 1930. The soil fungi of the Dovey Salt Marshes. Ann. Appl. Biol. 17:284-305.
- Fell, J.W., and I.M. Master. 1973. Fungi associated with the degradation of mangrove (Rhizophora mangle L.) leaves in south Florida, p. 455- 465. In L.H. Stevenson and R.R. Colwell (Eds.) Estuarine microbial ecology. Univ. South Carolina Press, Columbia.
- Gessner, R.V., and R.D. Goos. 1973a. Fungi from decomposing Spartina alterniflora. Can. J. Bot. 51:51-55.
- Gessner, R.V., and R.D. Goos. 1973b. Fungi from Spartina alterniflora in Rhode Island. Mycologia 65:1296-1301.
- Halliwell, G. 1963. Measurement of cellulose and factors affecting its activity, p. 71-92. In E.T. Reese (Ed.) Advances in enzymatic hydrolysis of cellulose and related materials. Pergamon Press, Oxford. ·
- Höhnk, W. 1957. Fortschritte der marinen Mykologie in jüngster Zeit. Naturwissenschaftliche Rundschau, Abt. 2, p. 39-44.
- Johnson, T.W., Jr. 1968. Saprobic marine fungi, p. 95-104. In G.C. Ainsworth and A.S. Sussman (Eds.) The Fungi, Vol. III. Academic Press, New York.
- Johnson, T.W., Jr., and F.K. Sparrow, Jr. 1961. Fungi in oceans and estuaries. J. Cramer, Weinheim. 668 p.
- Jones, E.B.G. 1968. The ecology and rotting ability of marine fungi, p. 237-258. In E.B.G. Jones and **S.K.** Eltringham (Eds.) Marine borers, fungi and fouling organisms of wood. Organisation for Economic Co-operation and Development, (OECD} Paris.
- Kazama, F., and M.S. Fuller. 1970. Ultrastructure of Porphyra perforata infected with Pythium marinum, a marine fungus. Can. J. Bot. 48: 2103-2107.
- Kirby, C.J., Jr. 1971. The annual net primary production and decomposition of salt marsh grass Spartina alterniflora Loisel. in the Barataria Bay estuary of Louisiana. Ph.D. Thesis, Louisiana State Univ. 74 p. ·
- Mendelssohn, I.A. 1973. Angiosperm production of three Virginia
marshes in various salinity and soil nutrient regimes. Masters Thesis, College of William and Mary. 103 p.
- Meyers, S.P., M.L. Nicholson, J. Rhee, P. Miles, and D.G. Ahearn. 1970. Mycological studies in Barataria Bay, Louisiana, and biodegradation of oyster grass, Spartina alterniflora. Coastal Studies Bulletin (Louisiana State Univ.) 5:111-124.
- Newell, S.Y. 1973. Succession and role of fungi in the degradation of red mangrove seedlings, p. 467-480. In L.H. Stevenson and R.R. Colwell (Eds.) Estuarine microbial ecology. Univ. South Carolina Press, Columbia.
- Odum, E.P. 1971. Fundamentals of ecology. 3rd ed. W.B. Saunders Co., Philadelphia. 574 p.
- Odum, E.P., and A.A. de la Cruz. 1967. Particulate organic detritus in a Georgia salt marsh estuarine ecosystem, p. 383-388. In G.H. Lauff (Ed.) Estuaries. Amer. Assoc. Advance. Sci. Publ. No. 83, Washington, D.C.
- Park, D. 1972. Methods of detecting fungi in organic detritus in water. Trans. Brit. Mycol. Soc. 58:281-290.
- Pettersson, G., and J. Porath. 1966. A cellulolytic enzyme from Penicillium notatum, p. 603-607. In E.F. Neufeld and V. Ginsburg (Eds.) Methods in enzymology, Vol.VII. Academic Press, New York.
- Pirozynski, **K.A.** 1968. Geographical distribution of fungi, p. 487-504. In G.C. Ainsworth and A.S. Sussman (Eds.) The Fungi, Vol. III. Academic Press, New York.
- Rautela, G.S., and E.B. Cowling. 1966. Simple cultural test for relative cellulolytic activity of fungi. Appl. Microbiol. 14: 892-898. .
- Scharpe, S., A. Lauwers, W. Cooreman, and W. Sierens. 1973. Viscometric assay of fungi cellulases with hydroxyethyl cellulose as substrate. Société Belge de Biochimie 81:982.
- Shearer, C.A. 1972. Fungi of the Chesapeake Bay and its tributaries. III. The distribution of wood-inhabiting Ascomycetes and Fungi .Imperfecti of the Patuxent River. Amer. J. Bot. 59:961-969.
- Siepman, R. 1959a. Ein beitrag zur saprophytischen pilzflora des Wattes der **Wesermundung.** I. Systematischer teil. Veroffentl. Inst. Meeresforsch., Bremerhaven 6:213-281.
- Siepmann, R. 1959b. Ein beitrag zur saprophytischen pilzflora des Wattes der Wesermündung. II. Veröffentl. Inst. Meeresforsch., Bremerhaven 6:283-301. ·
- Siu, R.G.H. 1951. Microbial decomposition of cellulose. Reinhold Pub. Corp., New York. 531 p.
- Sparrow, F.K., Jr. 1968. Ecology of freshwater fungi, p. 41-93. In G.C. Ainsworth and A.S. Sussman (Eds.) The Fungi, Vol. III. Academic Press, New York.
- Squiers, E.R., and R.E. Good. 1974. Seasonal changes in the productivity, caloric content, and chemical composition of a population of salt-marsh cord-grass (Spartina·alterniflora). Chesapeake Science 15:63-71.
- Stanier, R.Y. 1953. Adaptation, evolutionary and physiological; or Darwinism among the micro-organisms. Symp. Soc. Gen. Microbial. $3:1-22.$
- Strickland, J.D.H., and T.R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Bd. Canada Bull. 167, Ottawa. 311 p.
- Tansey, M.R. 1971. Agar-diffusion assay of cellulolytic ability of thermophilic fungi. Arch. Mikrobiol. 77: 1-11·.
- Thayer, G.W. 1974. Identity and regulation of nutrients limiting phytoplankton.production in the shallow estuaries near Beaufort, N.C. Oecologia (Berl.) 14:75-92.
- Wicklow, D.T. 1973. Microfungal populations in surface soils of manipulated prairie stands. Ecology 54:1302-1310. \mathbf{r}

Cruz, **A.A.** de la. 1965. A study of particulate organic detritus in a Georgia salt marsh-estuarine ecosystem. Ph.D. Dissertation, Univ. of Georgia. 110 p.

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Figure 1. Location of the sampling stations.

Figs. 2-5. Hydrographic characteristics of Stations H, M, C and W, . respectively.

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Figs. 6-9. Seasonal occurrence of phycomycetes at Stations H . (Pig. 6), M (Fig. 7), C (Pig. 8) and W. (Fig. 9).

Figure 6

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Figure 8

Table I

Taxa of fungi isolated at Stations H, M, c, W, and F and the number of isolates of each taxa at the various sampling periods. See Materials and Methods section for explanation of station designations.

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Table II

.Comparison of taxa. isolated at the various stations.

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Table III

Total number of taxa isolated below 10 C, above 11 C and the ratios of isolates/taxa at

the various stations.

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 $\hat{\mathcal{L}}_{\text{max}}$ and $\hat{\mathcal{L}}_{\text{max}}$

Table IV

Total numbers and percentages of organisms isolated at each station which were capable of clearing cellulose at any salinity.

92

 $\sim 10^6$

Table V

Tabulation of isolates capable of clearing cellulose at 0 , 15 and 30% at each station.

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Table VI

Tabulation of the number of genera capable of clearing cellulose at 0 , 15, and 30% at the various stations.

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Table VII

In situ elaboration of cellulolytic

enzymes at Station M.

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Table VIII

Range of organisms isolated on the various types of media (see Methods)

Table IX

Effect of various sources¹ of nitrogen and KH_2PO_4 on the C_χ enzyme activity (viscometric determination) of two isolates of frichoderma koningii Oud. *aggr.*

 1 All chemicals were added at a concentration of 0.4 g/l of medium. Growth medium: 1% finely ground Spartina; seawater (30% salinity) and pondwater (0% salinity); and the appropriate chemical.

² Relative activity = 100 (t₅₀)⁻¹ (ml culture fluid)⁻¹; t₅₀= time in min required for SO% reduction in viscosity of carboxymethyl cellulose solution. Reaction mixture: 1.2% Carboxymethyl cellulose (Hercules Powder Co., Type 7MF) in 0.05 M Sodium citrate nuffer at pH 5.6. Cannon-Fenske viscometric tubes #300 charged as recommended by manufacturer (about *T.O* ml). One milliliter of enzyme solution was utilized. Incubation temperature was 30 c.
Table X

Comparison between viscometric and agar diffusion assays in the detection of cellulolytic activity.

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Table XI

Comparison of carboxymethyl cellulose

activity of Trichoderma spp. by the viscometric and DNS methods.¹

¹ Growth medium: finely ground Spartina alterniflora, 10 g; urea, bactopeptone, KH₂PO₄, (NH₄)₂SO₄, 0.4 g; 1 liter of seawater (for 30%),
pondwater (for 0%) or seawater-pondwater 1:1 (for 15%) salinity. Harvest after 5 days of shake culture at 20 C.

2 See Materials and Methods.

3 Viscometric method (see Materials and Methods).

⁴DNS method (see Materials and Methods).

Appendix I

Tabulation of fungal isolates and the depth (mm) to which each isolate cleared cellulose at 0 , 15, 30% in the agar diffusion assay. Results reported as (*) indicate those organisms that grew to the depth indicated and made it difficult to determine the actual depth to which cellulolysis occurred, (t) signifies questionable clearing, while 'no data' indicates organisms which were lost or contaminated with other organisms.

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