FUNGAL FLORA OF A COVE ON LAKE FALCON

THESIS

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By

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ABSTRACT

A 12 month study of a cove in the riverine end of Falcon Lake was conducted to identify the fungal flora associated with submerged plant substrates. The substrates included stems of *Arundo donax*, *Prosopis glandulosa*, and *Salix nigra* which were collected monthly. The leaves of *Salix nigra* were also collected monthly for observed Aquatic Hyphomycetes. A measurement of the fungal biomass on *Arundo donax* would be determined by a survey of numbers of ascoma on the stems. The dominant fungi that colonized the stems were *Leptosphaeria tetonensis*, its anamorph *Phoma*, and *Didymosphaeria sp.* The Aquatic Hyphomycetes *Alatospora* and *Lunulospora* appeared seasonally on *Salix* leaves. The heaviest colonization of the culms of *Arundo* was seen in May. The observed dominance of some species probably results from factors such as pH, oxygen concentration and inter-species competition.

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INTRODUCTION

In the spring of 1992 a study was initiated to study the fungal populations in a cove at the riverine end of Falcon Lake. The study site is located 26 miles southeast of Laredo, Texas near San Ygnacio in Zapata county. The cove is unique in that it has a sand bar which helps to maintain a fairly constant level of water even when Lake Falcon recedes. The arroyo Zopilote, an intermittent stream, feeds into the cove opposite Falcon Lake, and therefore helps maintain water levels in the cove. In 1984-1986, because of agricultural pumping and low rainfall, Falcon Lake dramatically receded and the cove dried (International Boundary and Water Commission Bulletins 54, 55, 56, 1984-1986). This promoted the growth of many native woody and herbaceous plants in the dry cove bed. *Arundo donax* "giant river reed", *Prosopis glandulosa* "honey mesquite", and *Salix nigra* "common black willow" were the most abundant. In 1988 the lake flooded these populations of plants, thus providing a massive input of woody debris upon which fungal communities could thrive.

The role of aquatic fungi, especially aquatic hyphomycetes is an indispensable part of the aquatic ecosystem (Ingold, 1960; Barlocher & Kendrick, 1974, 1976; Michaelides & Kendrick, 1978; Barlocher, 1985). Certain representatives of Ascomycetes, Oomycetes, Hyphomycetes, and Deuteromycetes are known to decompose and recycle allochthonous woody and leaf material. This is accomplished by the use of a variety of digestive enzymes secreted by the fungi. Wood is composed of 40-60% cellulose, 10-30% hemicellulose, and 15-30% lignin and it is acted upon by a variety

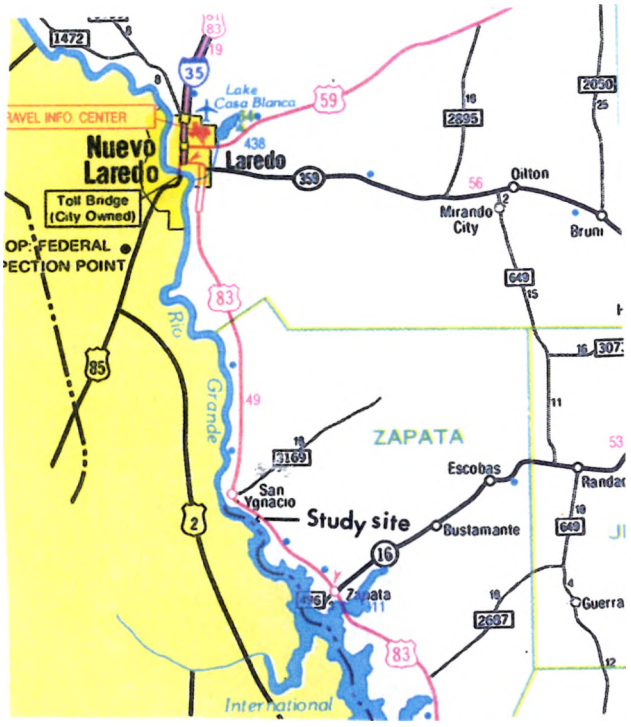

Figure 1. Map of south Texas showing San Ygnacio Texas and the approximate location of the study site.

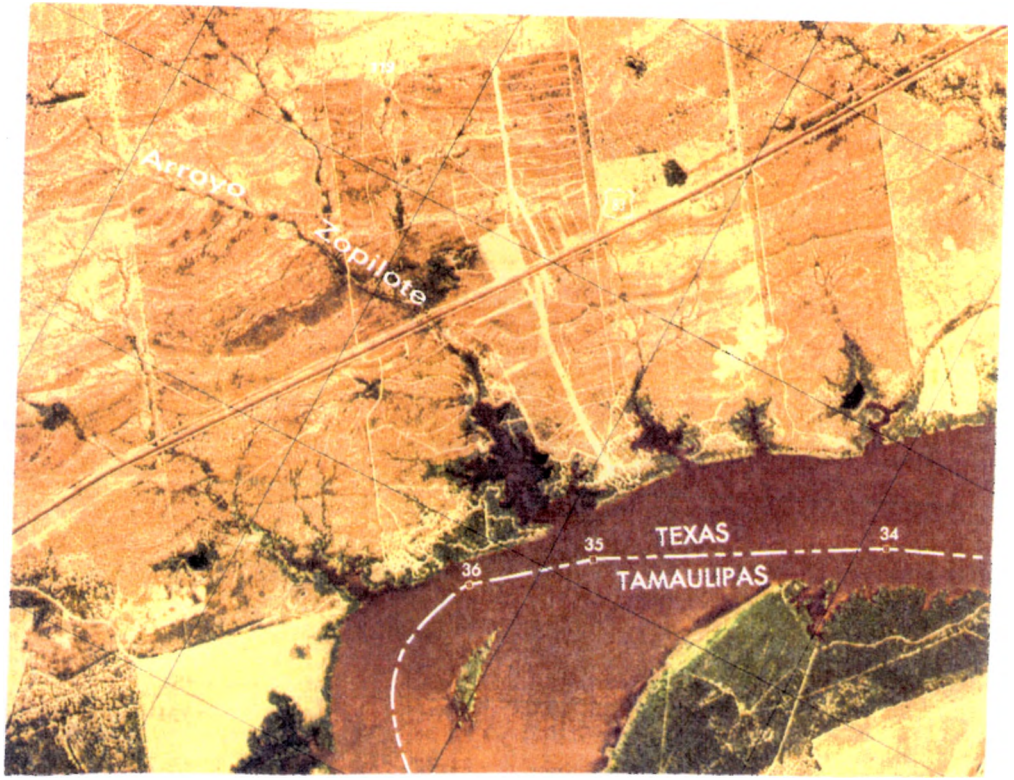

Figure 2. Aerial photograph of the cove and Rio Grande taken in 1982. The lake is at 2,627 thousand acre feet (United States Geological Survey 1982).

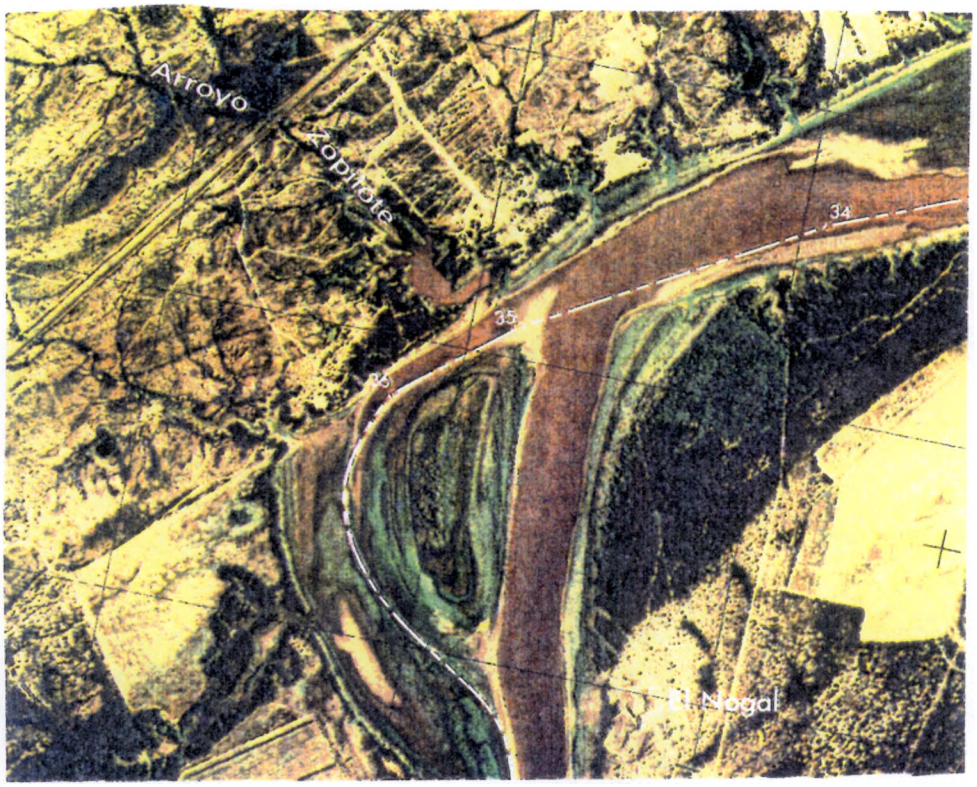

Figure 3. Aerial photograph of the cove and Rio Grande taken in 1983. The lake is at 1,246 thousand acre feet (United States Geological Survey 1983).

Figure 4. Populations of *Arundo donax* which surround the cove.

Figure 5. Populations of *Salix nigra* which are flooded in and around the cove.

Figure 6. The *Prosopis glandulosa* which inhabit the cove edge.

of extracellular enzymes. The cove has slightly basic water which some researchers suggest can promote increased fungal enzyme activity (Chamier & Dixon, 1983; Kok, Haverkamp & Van der Aa, 1992; Suberkropp & Klug 1980; Suberkropp, 1992b) and conidium production of aquatic hyphomycetes (Barlocher, 1982). Wood is processed by fungi often resulting in production of soft rots (Hudson, 1986): or erosion troughs (Jones, 1981) . Through this digestive processing, the wood and leaf material then can be used as a food source for a variety of aquatic organisms. Aquatic fungi inhabiting plant substrates have been found to function as a dietary supplement for aquatic invertebrate shredders (Barlocher & Kendrick, 1976; Cummins & Klug, 1979; Barlocher, 1985; Arsuffi & Suberkropp, 1989; Suberkropp 1992a, 1992b).

Many studies have been performed on the role of aquatic fungi and their contribution to the overall aquatic ecosystem. Work by Cummins & Klug, (1979) has shown that invertebrate shredder detritus assimilation into body weight is encouraged by feeding upon leaves inhabited by microbes and especially fungi. Shredders will usually prefer or even select leaves colonized by fungi, and will usually feed upon such leaves only when the colonization is at a maximum (Cummins & Klug, 1979). Conditioning of leaves by aquatic hyphomycetes has been shown to increase the palatability of leaves (Barlocher & Kendrick, 1975), and that aquatic invertebrate shredders have been shown to have a preference for certain leaves and the specific species of fungi which colonize them (Arsuffi & Suberkropp, 1985, 1989; Barlocher, 1985). The waste and food particles produced by these first feeding groups will provide food for other invertebrates with differing feeding strategies, thus driving a food chain (Cummins & Klug, 1979). The

main allochthonous substrates are wood, twigs and leaves, each of which have been shown to support widely different populations of fungi.

One of the main roles of woody debris is to provide a stable environment with a long residence time for fungal communities. Larger debris such as logs change the morphology and flow of a river (Swanson & Lienkeamper 1976). Wood acts as a permanent food source upon which different fungal organisms may depend throughout the year. This is especially important for Ascomycetes which require a longer time for completion of life cycles (Shearer & Von Bodman, 1983; Shearer, 1992). Leaves usually degrade quickly (Petersen & Cummins, 1974) and do not provide enough time necessary for production of ascomata. Therefore, members of the imperfect and lower fungi are common saprophytes of leaves (Kohlmeyer & Kohlmeyer, 1979). Teleomorphs of many Deuteromycetes including the Aquatic Hyphomycetes are commonly found on submerged wood (Shearer, 1989). Woody substrates therefore act as a reservoir for Aquatic Hyphomycetes in the aquatic habitat, so when leaf material becomes available it may become quickly inoculated. Studies by Shearer and Webster showed that Aquatic Hyphomycete community composition on wood reflects the community structure of the fungi on leaves (Shearer & Webster, 1991). Wood can also act as a dispersal agent for Aquatic Hyphomycetes and other fungi since logs and twigs can travel long distances in a stream or river and occasionally survive to reach the sea (Shearer & Von Bodman, 1983; Shearer, 1992). Many occurrences of Aquatic Hyphomycetes in brackish and saltwater have been reported (Jones & Oliver, 1964; Kirk, 1969; Shearer & Crane, 1971; Shearer, 1972; Muller-Haeckel & Marvanova, 1979; Oxley 1992). Many ascomycetes common in brackish or salt water habitats are also believed to have

freshwater ancestors which were transported to the marine environment (Shearer & Von Bodman, 1983).

In this study, the fungal communities which inhabit the three main substrates *Arundo, Prosopis*, and *Salix* were examined. The goal was to see if any one class of fungi had a specific preference for any of the three substrates. *Prosopis* and *Salix* are dicots, while *Arundo* is a monocot. Varying components of lignin, cellulose, pectin, and hemicellulose, should differentiate the community structure on each of these substrates based on the ability of fungi from the aquatic habitat to process these compounds. Also, fungal biomass inhabiting *Arundo* would be determined.

The Rio Grande River which feeds Falcon Lake is a major source of water for border communities in northern Mexico and South Texas. It is also a heavily polluted river, a fact which has undoubtedly caused severe damage to the organisms and ecology of this river. This project offered a unique opportunity to study the aquatic fungal communities on the Rio Grande River; a river which has never been studied for mycological populations.

METHODS AND MATERIALS

Before the actual study proceeded, a three month preliminary study was conducted to test the feasibility of using this particular study site and determine the best methods to be implemented in subsequent studies. In this testing period the physical parameters of the cove water including dissolved oxygen, salinity, pH, and temperature were measured. This preliminary study period included a study of submerged plant substrates from which identification of the fungal communities was carried out.

A twelve month study beginning in May 1992 was then initiated to allow for measurement of myco-floral diversity, substrate preference, annual myco-floristic fluctuation, and fungal biomass/density. The study was limited to the riverine cove on Lake Falcon. Monthly collection of submerged substrates, and measurement of physical parameters of the cove water was performed. The submerged substrates included the stems of the dicots *Salix nigra*, and *Prosopis glandulosa*, and the monocot *Arundo donax*. Submerged leaves of *Salix nigra* were collected for examination of aquatic hyphomycete populations. Ten pieces of the woody and monocot substrates respectively were collected and transported to the mycology laboratory at SWTSU. There, 15 cm pieces with an average diameter of 3 cm were then randomly cut from each for examination and identification of fungi. For each 15 cm piece of material, a detailed surface scan allowed for observation of fungal saprophytes. Upon location of fungal soma or reproductive structures, they were removed, observed microscopically and

identified. A representative of each genus was preserved in lactophenol on microscope slides to act as a floral library for future reference.

Submerged leaves were likewise collected and returned to the laboratory where eight, 4 mm plugs were cut from each leaf using a cork borer. Eight leaf plugs were placed in each of three, 125 ml Erlenmeyer flasks with 50 ml of filtered, buffered, de-ionized water. Three such flasks were made each month to identify aquatic hyphomycetes which might inhabit the leaves. The three individual flasks were placed on a shaker for a 72 hour period to allow for aeration and development of conidia. After shaking, the water in each then was filtered through a Millipore filter apparatus using filters of 5 microns to collect the dislodged conidia. Following the filtration process a rinse with water followed by a 0.1 % lactophenol/ toluidine blue stain solution in order to stain and more easily observe the conidia. Each stained filter with its conidia was mounted on a 2.5 cm X 7.5 cm glass slide, using a 0.1% lactophenol solution which caused the filters to become transparent. Each slide was then observed for conidia of hyphomycetes and identification was made with the use of hyphomycete keys (Nilsson, 1964; Ingold, 1975). The number of conidia and species dislodged in the 72 hour period was determined by counting the number of conidia observed in a randomly selected 6 mm plug of the filter cut with a cork borer. This experiment was designed in triplicate. Calculation of the number of conidia produced over the 72 hour period was accomplished by the following formula.

$$\frac{Y}{.848cm} = \frac{X}{18.1cm}$$

.848 = Area of selected filter paper plugs.

18.1 = Area of the filter paper.

Y = Number of conidia counted on all three filter plugs.

X = Number of conidia existing on the entire filter paper.

Additionally, a survey of the oomycetes present in the cove was initiated. Each month 2 cm X 4 cm mesh bags containing baits of hemp seeds and mung beans were placed in the water for collection in the following month. Whole seeds allowed for longer survival in the water, and adequate colonization. Collected bags were taken to the SWTSU laboratory where they were opened and the contents transferred to distilled water and incubated with fresh seeds for a period of 4 days. Any observed fungal growth was examined microscopically and identified.

The physical parameters of the water were determined monthly using La Motte kits for determination of dissolved oxygen and salinity. A pocket pH meter was used to determine pH; readings were taken in both the cove and the river. Water for these tests was taken at a depth of 30 cm. Temperature was taken with a Celsius thermometer submerged in water no less than 30 cm.

Three 2.5 cm squares of flattened *Arundo* stem tissue were randomly selected to determine ascomal density. Each square was examined for ascomata with a stereo microscope, and the number per square centimeter was determined from the average of three specimens. Ascomal density was calculated and used to indicate possible fungal biomass in the plant tissues.

RESULTS

Physical Parameters of The Cove Water

The pH of both the cove and river water (Figure 7) remained slightly basic throughout the twelve month study period. During the study, the cove water remained slightly more acidic than the river water. Oxygen content of the cove water was measured and ranged from 2 parts per million to 12.3 ppm and had an average of 6.5 ppm (Figure 8). These results fit well with the temperature recorded over the twelve months (Figure 10). The oxygen content of the water fluctuated with fluctuation in temperature thus reflecting the ability of the water to absorb oxygen. The salinity content of the water remained fairly constant throughout the study period. In figure 9, the salinity ranged from 0.6 parts per thousand to 2 ppt over the study period.

Fungal Classes Isolated from Substrates

The three surveyed substrates *Arundo donax*, *Salix nigra*, and *Prosopis glandulosa* yielded a total of 31 taxa of fungi (Table 1). Tables 2-8 have commonly occurring species on the substrates listed in red print. A total of eight Ascomycetes were isolated from *A. donax* (Table 2). The two dominant Ascomycetes were *Didymosphaeria sp.* occurring in all twelve months, and *Leptosphaeria tetonensis* occurring in all months except July. Genera of *Leptosphaeria* are well represented in this cove on *A. donax.* Besides *L. tetonensis* an additional 3 species *L. erigerontis, L. discors, and L. staritzii* were isolated from *Arundo* and occurred infrequently during the study as did three other genera of Ascomycetes.

S. nigra also supported *L. tetonensis* as a dominant member of its mycoflora, but it had fewer total Ascomycetes than *A. donax*. *Didymosphaeria sp.* also was fairly common appearing along with two other infrequent Ascomycetes *L. staritzii* and *Pleospora* (Table 3).

P. glandulosa supported seven species of Ascomycetes (Table 4). Again, *Didymosphaeria sp.* was seen as a dominant community member occurring in all months except May and July while *L. tetonensis* was present only occasionally. Four other Ascomycetes along with *L. discors* were also isolated from *P. glandulosa*.

Six Deuteromycetes were isolated from the culms of *A. donax*, but only one existed as a dominant community member for all twelve months (Table 5). *Phoma* occurred in 7 out of 12 months while all others had a very spotty distribution from September to February. *S. nigra* exhibited similar results in having *Phoma* as a dominant member of the fungal community along with 8 other genera (Table 6). *P. glandulosa* also had *Phoma* as a dominant community member, but it had eight other genera which were less frequent and scattered in their appearance (Table 7). The total number of species on each substrate is shown in bar graph form to illustrate the most fungally hospitable substrate (Figure 11). As the bar graph indicates *A. donax* seemed to be the most heavily colonized substrate. *P. glandulosa* also seems to be a preferred substrate having almost as many species as *A. donax*.. *S. nigra* seems to become a more available substrate in October through May for the fungal community to colonize.

Oomycetes isolated from baited traps

As Table 8 indicates only two Oomycetes were isolated from the waters of the cove. *Saprolegnia* was identified in all months except June, and *Dictyuchus* was found in May, October, January, and August (Table 8).

Aquatic Hyphomycetes Isolated from *Salix nigra* Leaves

The number of conidia per square centimeter of leaf material produced in each month from collected *S. nigra* leaves is shown in Figure 12. The presence of colonized leaves is infrequent from May to November, but in December a massive bloom of *Alatospora* is seen. The populations persist from February through April. *Lunulospora* also produces a large bloom, but it comes one month later in January along with the appearance of *Tetracladium* while it later subsides in February when *Anguillospora* also appears (Figure 12).

Fungal biomass observed on *Arundo donax*.

Figure 13 shows the numbers of ascoma produced on the submerged culms of Arundo per square centimeter. May and July exhibit the heaviest colonization of the culms, and this trend of heavy colonization continued through November (Figure 13).

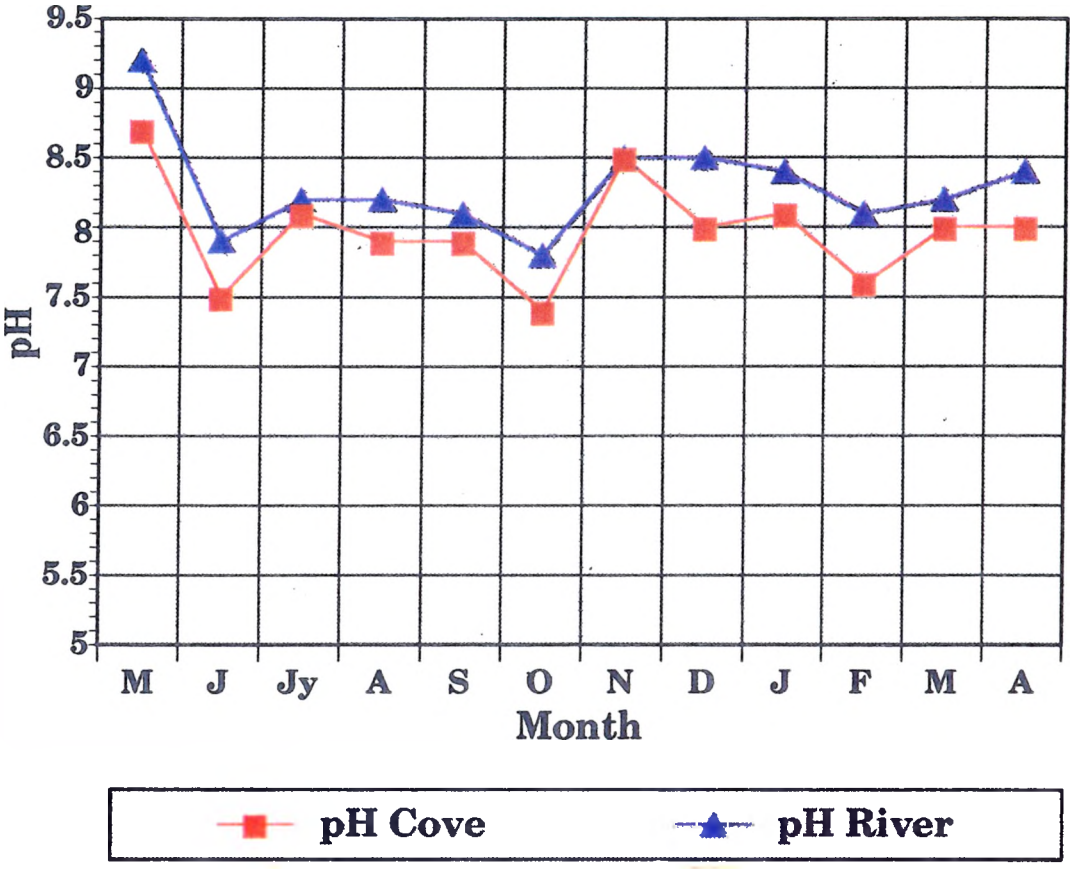

Figure 7. The pH of the water in the cove and the river.

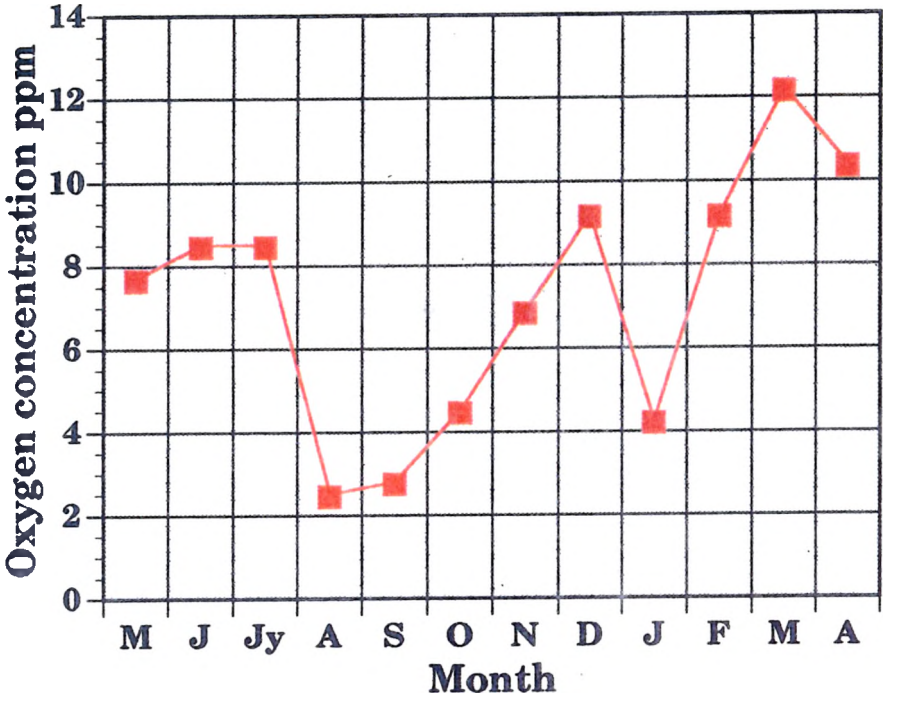

Figure 8. Oxygen content of the cove water in parts per million.

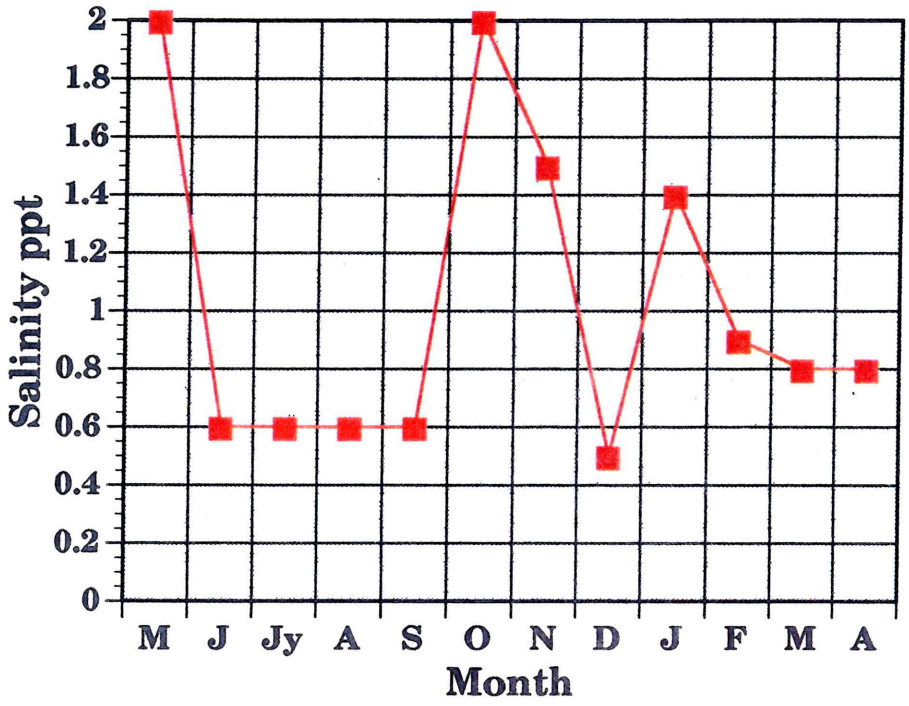

Figure 9. Salinity of the cove water in parts per thousand.

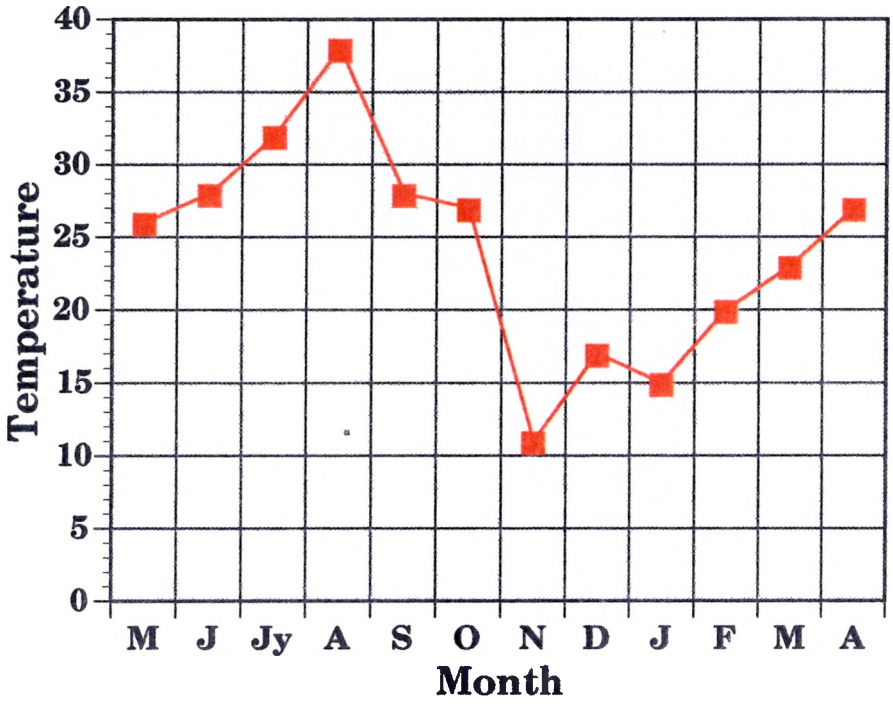

Figure 10. Temperature of the cove water in °C.

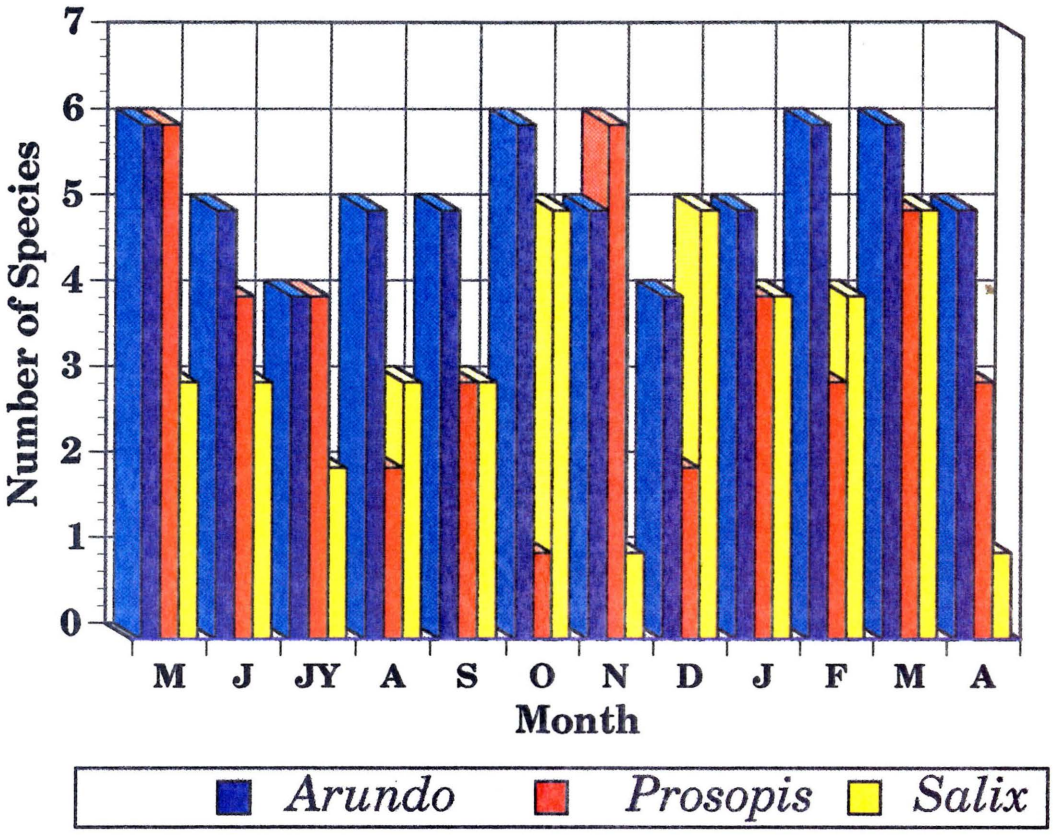

Figure 11. Species richness on *Arundo donax*, *Salix nigra*, and *Prosopis glandulosa* collected from the cove.

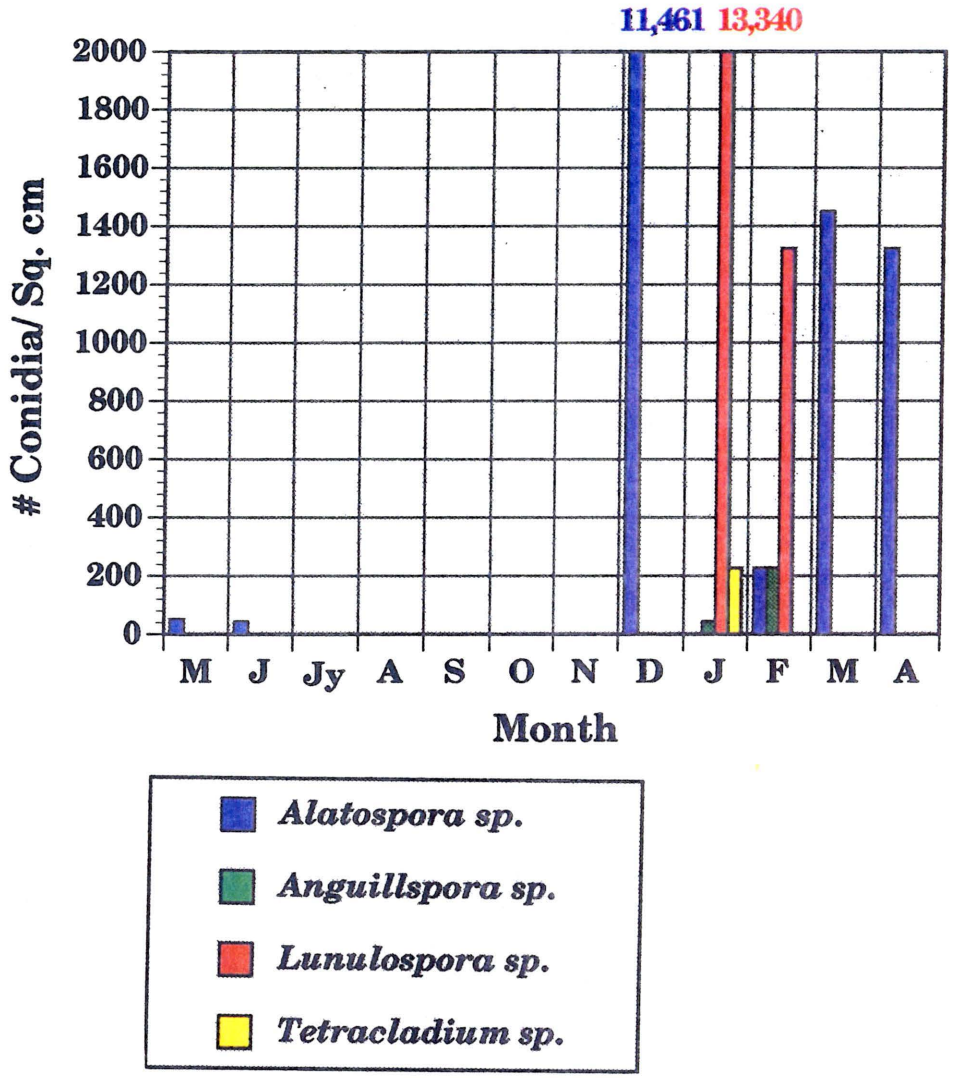

Figure 12. Aquatic Hyphomycetes isolated from *Salix nigra* leaves.

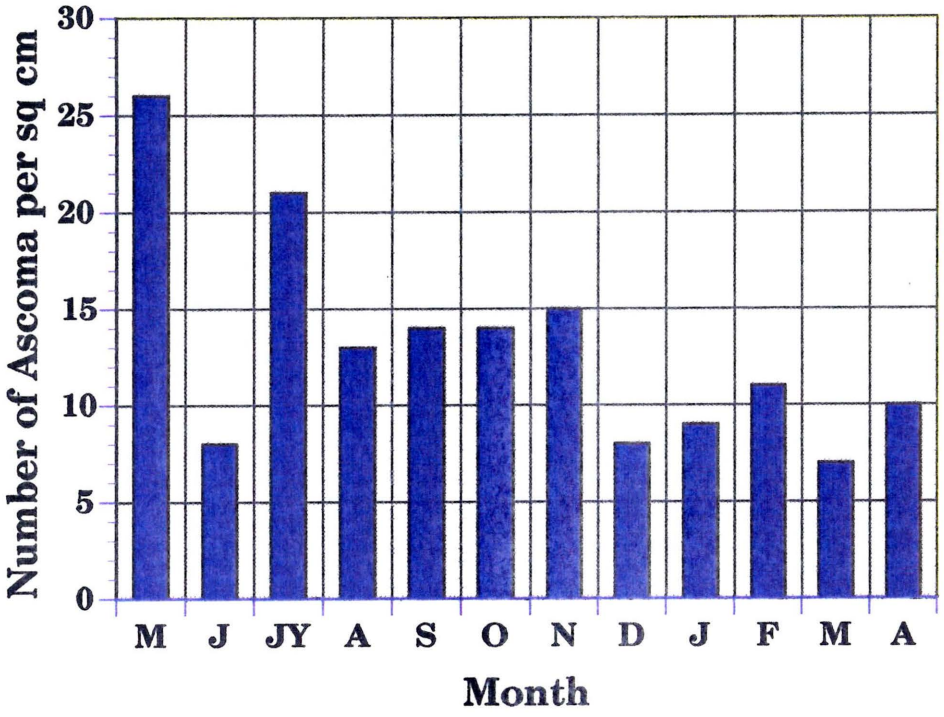

Figure 13. Ascoma production per square centimeter on *Arundo donax*.

Table 1. Fungi isolated from the cove.

Substrates	*Arundo*	*Salix*	*Prosopis*
Deuteromycetes			
Anguillospora Ingold.		X	
Alatospora Ingold.		X	
Alternaria Nees.	X	X	X
Aposphaeria Sacc.		X	
Botrydiplodia Sacc.			X
Coniothyrium Sacc.			X
Diplodia Fr.			X
Diplodina Westend.		X	
Macrophoma Berl & Vogl.		X	
Lunulospora Ingold		X	
Penicillium Link.			X
Phoma Desm.	X	X	X
Prosthemium Kunze			X
Rhyniophoma Karst		X	
Stagonospora Sacc.	X		
Taeniolella Hughes	X	X	X
Tetraploa Berk & Vr.	X	X	
Tetracladium Ingold		X	
Trichoderma Pers.	X	X	X
Ascomycetes			
Didymella Sacc.	X		
Didymosphaeria Fuckel X	X		X
Cochliobolus Drechs.		X	
Gaeumannomyces Arx & Oliver	X		
Hysterographium Corda			X
Leptosphaeria erigerontis Berlese	X		
L. discors. Sacc.	X		X
L. tetonensis Ellis & Everhart X	X		X
L. Staritzii Rhem	X		X
Pleospora Rabenh.		X	X
Pyrenophora Fr.	X		
Xylaria J. Hill			X
Oomycetes Identified from hemp seeds			
Saprolegnia Nees			
Dictyuchus Leitgeb			

Table 2. Ascomycetes isolated from *Arundo donax*.

Species / Month	M	J	Jy	A	S	O	N	D	J	F	M	A
Didymella						X						
Didymosphaeria	X	X	X	X	X	X	X	X	X	X	X	X
Gaeumannomyces				X								
Leptosphaeria erigerontis							X					
L. discors						X		X				
L. tetonensis	X	X		X	X	X	X	X	X	X	X	X
L. staritzii	X					X				X		
Pyrenophora	X		X									

Table 3. Ascomycetes isolated from *Salix nigra*.

Species / Month	M	J	Jy	A	S	O	N	D	J	F	M	A
Didymosphaeria	X	X		X							X	X
Leptosphaeria tetonensis	X		X	X	X	X	X	X	X	X	X	X
L. staritzii						X						
Pleospora								X	X			

Table 4. Ascomycetes isolated from *Prosopis glandulosa*.

Species / Month	M	J	Jy	A	S	O	N	D	J	F	M	A
Didymosphaeria		X		X	X	X	X	X	X	X	X	X
Cochliobolus			X									
Hysterographium	X											
Leptosphaeria tetonensis			X				X			X		X
L. discors											X	
Pleospora								X	X			
Xylaria	X					X						

Table 5. Deuteromycetes isolated from *Arundo donax*.

Species / Month	M	J	Jy	A	S	O	N	D	J	F	M	A
Alternaria							X					
Phoma	X	X		X	X		X		X	X		
Stagonospora						X	X					
Taeniolella							X		X			
Tetraploa					X							
Trichoderma							X		X			

Table 6. Deuteromycetes isolated from *Salix nigra*.

Species / Month	M	J	Jy	A	S	O	N	D	J	F	M	A
Alternaria					X							
Aposphaeria	X										X	
Diplodinia											X	
Macrophoma										X		
Phoma	X	X	X	X	X	X	X	X	X	X	X	X
Rhyniophoma		X										
Taeniolella										X		X
Tetraploa						X						
Trichoderma		X							X	X		

Table 7. Deuteromycetes isolated from *Prosopis glandulosa*.

Species / Month	M	J	Jy	A	S	O	N	D	J	F	M	A
Botryodiplodia	X											
Coniothyrium								X				
Diplodia	X	X										
Macrophoma					X			X				
Penicillium		X				X					X	
Phoma	X	X		X	X			X		X	X	X
Prosthemium	X											
Taeniolella								X				
Trichoderma			X						X	X	X	

Table 8. Oomycetes isolated from hemp seeds in mesh bags.

Species / Month	M	J	Jy	A	S	O	N	D	J	F	M	A
Saprolegnia	X		X	X	X	X	X	X	X	X	X	X
Dictyuchus	X					X			X			X

DISCUSSION

Freshwater decay of woody substrates by fungi is a subject which has received little in the way of research, as compared to marine Ascomycetes. A multitude of studies have been performed on leaf inhabiting Aquatic Hyphomycetes since their discovery by Ingold (1942). According to Zare-Maivan and Shearer (1988) much of our present knowledge of freshwater lignicolous fungi comes from published studies of wood slats from cooling towers (Eaton, 1976; Leightley & Eaton, 1977). Studies of lignicolous Ascomycetes from the aquatic habitat are scarce (Shearer & Von Bodman 1983). This study provided a unique opportunity to study lignicolous fungi in a near sub-tropical setting and identify some of the aquatic fungal flora associated with the southern portion of the Rio Grande.

The study of the cove on Falcon Lake was the first mycological study conducted in this environment. The one aspect that sets it apart from similar studies of aquatic fungi is the unique way the substrates were collected and eventually handled in the laboratory. Most mycological studies of submerged substrates allows for an incubation period of six months up to one year. This longer incubation allows for the growth and sporulation of fungi, particularly Ascomycetes, which may lie dormant in the plant tissues. This method yields the greatest number of species and allows for a greater frequency of individual species (Shearer & Von Bodman 1983). The substrates sampled for fungal flora in this study represented fungi which were actively growing and sporulating. The data collected from each individual piece of substrate accurately portrays the fungal

community that is active on it at the time of collection. This method of handling and examining substrates eliminates several problems which can distort a researchers' data. Metabolic products produced by growing fungi, improper maintenance of moisture levels, and possible contamination of collected substrates with a foreign species of fungi can all affect or inhibit the growth and sporulation of normal flora thus making the portrayal of the actual community difficult.

Also unique to this study is the method of the survey of the Aquatic Hyphomycete flora on the submerged leaves. The method used is a hybrid of two commonly used methods of hyphomycete collection and identification. The method of leaf sampling is one which has been shown to be highly successful, and accurate in determining the hyphomycete community on leaves. This method basically requires the collection of leaves and a short incubation period followed by direct examination of the leaf surface. Filtration is a quick and easy method to sample and quantify Aquatic Hyphomycetes present, but has been shown to introduce many problems. Problems include changes in conidia morphology, similarities in conidia, and overlapping of conidia.(Shearer & Lane, 1983). The method used in this study takes the aspects of both these methods which produced accurate and easily repeatable results.

The chemistry of a body of water can affect the occurrence and frequency of aquatic fungi. So close attention was paid to the physical nature of the cove water. The cove water remained fairly basic ranging from 9.2 to 7.4 (Figure 7). Alkalinity of water has been shown to influence what types of fungi occur and the total numbers of those fungi. It has been shown that many fungi, especially Aquatic Hyphomycetes prefer circumneuteral waters with a pH 5.7 to 7.2 (Chamier 1992, Barlocher 1987,

Barlocher & Rosset 1981). Alkalinity has been found to enhance the conidium production of Aquatic Hyphomycetes (Barlocher 1982). Here, alkalinity could be a major factor in the dominance of some Ascomycetes over others. As seen in Tables 1, 2, 3, the ascomycetes *Didymosphaeria* and *Leptosphaeria tetonensis* dominate the fungal flora based on observed frequency. Perhaps these species are more tolerant to the relative alkalinity of the cove water thus making them more successful in this habitat. Dominance of a species on the three substrates was also seen in tables 4, 5, 6, with the Deuteromycete, *Phoma,* a possible anamorph of *Leptosphaeria sp.* This corresponds well with the regular appearance of various species of *Leptosphaeria.* Alkalinity of the cove water also may play a role in the low number of individual Aquatic Hyphomycete species isolated from *Salix* leaves as seen in Figure 12. The alkalinity of the water, as has been suggested, will limit a diversity of individual species of Aquatic Hyphomycetes. *Alatospora* and *Lunulospora* are shown to be more successful than *Tetracladium* and *Anguillospora* in conidium production in this basic environment thus, making them dominant species when adequate leaf litter becomes available in the water.

Oxygen concentration of water was also monitored because of the affect that it might have on the fungi present. Aquatic fungi are generally all aerobic organisms and require adequate oxygenation to grow and sporulate correctly (E. B. G. Jones 1971). Fluctuation in dissolved oxygen concentration was seen in the water, but this fluctuation was most likely a reflection of the temperature of the water. As Figures 8 and 10 show, fluctuation in the water temperature had some effect on the oxygen concentration. Low oxygen concentration was also most likely a limiting factor on the Aquatic Hyphomycete species which can occur. Few Aquatic

Hyphomycetes have been observed in slow running or stagnant waters (Nilsson 1964). *Alatospora* has been shown by Koske & Duncan (1974) to have a sporulation temperature range of 15ºC- 25ºC which in a lotic environment can have a dramatic affect on the oxygen concentration of the water (Koske & Duncan 1974). As seen in Figure 12 conidium production from abscised leaves was highest in December, April and May suggesting prior colonization of the leaves in the cove was also at a maximum. In these months the water temperature was 15ºC in December, 27ºC in April, and 26ºC in May which nicely fits Koske and Duncan's results as these temperatures fall within the known maximum sporulation temperatures.

Salinity tolerance was also taken into consideration in the testing of the cove water. As seen in Figure 9, salinity fluctuated several times throughout the study but otherwise the water remained in a narrow range. The reason for the fluctuation is not clearly understood as the lake levels were high indicating little water loss because of evaporation or irrigation (International Boundary and Water Commission Bulletin 92, 1992). These occasional fluctuations of the salinity of the water didn't appear to have any effect on the fungal populations of this environment.

The substrates themselves also seemed to have some effect on the fungal community present. As Figure 11 shows, *Arundo donax* produced a greater number of species than did *Salix nigra* or *Prosopis glandulosa* in most months. This indicates that it was a commonly available substrate and, possibly due to the lack of tough lignified tissue common in woody dicots provided a much preferable substrate. *Prosopis* seemed to become a more preferred and available substrate in spring and summer while *Salix* was more preferred and available in fall and spring. In ascomycete diversity, *Arundo donax* had eight different ascomycetes associated with it

(Table 2). *Prosopis* also had a comparable number of ascomycetes, seven being isolated from it (Table 4). Table 3 shows that *Salix nigra* was less commonly colonized by Ascomycetes, having only four associated with it (Table 3). The ability of a substrate to support a more diverse fungal community depends on the nutrient quality of the substrate and the speed of how quickly nutrients are leached from it. It is known that soluble starches, sugars and proteins are quickly leached from twigs when they enter the water (Willoughby & Archer, 1973). So, the fungal community will change as leaching alters the nutrient value of the substrate. Another aspect to consider is the condition of the twigs at the time of collection and how long they have been in the water. The *Salix* twigs lacked bark and had undoubtedly been in the water for an extended period of time. Species of *Leptosphaeria* have been found to quickly colonize twigs after submersion and have been seen to increase in frequency with increased time of submersion (Lamore & Goos, 1978). However, the *Salix* wood supported *Leptosphaeria tetonensis* in high frequency every month throughout the year (Table 3). *Leptosphaeria tetonensis* also occurred commonly on *Prosopis* as well as *Arundo* (Tables 2 and 4). All *Prosopis* twigs had bark at collection and *Arundo*, being a monocot, had no bark or lignicolous secondary growth. With this information *Leptosphaeria tetonensis* can possibly be seen as a generalist due to its high frequency on widely varying substrates. A similar assumption can be made of *Didymosphaeria sp.* as it also occurred frequently on all observed substrates. The Deuteromycete, *Phoma* may also fall into this category of generalist, as it also occurs frequently on all three substrates.

The fungi occurring on the substrates themselves may have an inhibitory effect on other species because of the production of anti-fungal

agents. A study by Shearer and Zare-Maivan in 1988 exhibited the presence of anti- fungal agents produced by some aquatic fungi which can eliminate competitive species and thus reduce substrate competition pressure. Among the aquatic fungi which exhibited these anti fungal enzyme production capabilities were two species of *Leptosphaeria* (Shearer & Zare-Maivan 1988). Here is yet another possibility for the successful dominance of *Leptosphaeria tetonensis* seen on all three substrates. Perhaps this species of *Leptosphaeria* also exhibits this anti fungal activity described by Shearer and Zare-Maivan.

Of the Oomycetes observed from hemp seed baits, *Saprolegnia sp.* occurred most frequently throughout the study (Table 8). *Dictyuchus sp.* also occurred frequently but was not as prolific as *Saprolegnia*. This poses an interesting question of how polluted is the Rio Grande river? A study by Harvey in 1952 showed that oomycetes occur in good populations in non-polluted water but as pollution increased fewer and fewer oomycetes are seen. Also, where pollution from domestic sewage was present Oomycetes were rare (Harvey 1952). In the Rio Grande, pollution in the form of raw sewage is a real problem as towns in Mexico have inadequate sewage treatment and often add raw sewage to the waters of the river. This cove possibly offers some degree of protection to the Oomycetes from the effects of this urban and industrial pollution.

Ascoma production per square centimeter on *Arundo donax* was determined to establish the fungal biomass associated with the submerged substrates. This aspect of the study was performed to obtain some idea of the extent that *Arundo* was colonized. As seen in Table 8, the highest amount of fungal biomass was seen in May through November. This suggests that *Leptosphaeria tetonensis* and *Didymosphaeria*, the two

ascomycetes most commonly observed, heavily colonize *Arundo* in the summer and fall (Table 8).

The appearance of Aquatic Hyphomycetes over the study period also corresponded well with previous studies (Michaelides & Kendrick, 1978; Suberkropp 1984; Akridge & Koehn 1987). Here a seasonal distribution is shown and all four observed aquatic hyphomycetes occurred in conjunction with the leaf abcision from the trees. As Figure 12 reveals, little to no activity occurred from May to November. However, the period from December to April indicated that the fungi were highly active as reflected by the abundance of spores isolated from leaves in the water. The winter of 1992-1993 was particularly mild, with no heavy frost, thus the leaf fall was constant throughout winter and spring. This explains the extension of the hyphomycete populations into the spring season.

The lack of previous research on the Rio Grande makes this study the primary baseline for determining the aquatic fungal flora. The results show that this environment is extremely restrictive in that only three periodic and dominant species exist with all others occur sporadically. The effect pollution has on the fungal flora will have to be determined in subsequent studies. Another question to be asked is what effect will the pollution have on the microinvertibrate populations whose life cycles are interwoven with the success of the heterotrophic fungal community? The ecosystem most likely has and will continue to be damaged until dumping of sewage and industrial waste is halted by joint efforts of the United States and the Mexican Government.

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