Studies on *Alternaria* associated with salt marsh halophytes

Tawfik M. MUHSIN

Department of Biology, College of Education, University of Basrah, Basrah, Iraq

Summary. – Twelve species of *Alternaria* were isolated from six salt marsh halophytes, *Atriplex patula, Glaux maritima, Hordeum jubatum, Puccinellia nuttalliana, Salicornia europaea* and *Suaeda depressa*. Differences in species diversity, frequency percentage, occurrence, and dominance function were observed for the *Alternaria* species associated with the six plants. Succulent halophytes were found to differ in their fungal assemblage from the non-succulent plants. The differences in fungal colonization are discussed in relation to the nature of the plants.

Introduction

Many halophytic plants species are distributed in salt marshes over the world and are mostly dominating in graminoid, shrub and succulent plants (CHAPMAN, 1974; UNGAR, 1974). The majority of information available on salt marsh fungi is restricted to examination of rhizospheres of dead or living plants (APINIS & CHESTERS, 1964; DICKINSON, 1965; HENDERARTO & DICKINSON, 1984). PUGH (1974) and KOHLMEYER & KOHLMEYER (1979) have extensively reviewed the studies dealing with fungi on salt marsh halophytes. Apparently little is known about the ecology of mycoflora in salt marshes in general. GESSNER (1977) studied the seasonal occurrence and distribution of fungi on *Spartina alterniflora* LOISEL in salt marshes of the United States and recently MUHSIN (1985) has surveyed and analyzed the community of mycota on different halophytes, in response to edaphic and climatic factors, in salt marshes of Canada.

This study was undertaken to consider the composition of *Alternaria* assemblage on six different halophytes, viz. *Atriplex patula* L., *Glaux maritima* L., *Hordeum jubatum* L., *Puccinellia nuttalliana* (SCHUTT.) HITCH., *Salicornia europaea* agg., and *Suaeda depressa* (PURSH.) WATS., throughout the growing season. Fungal community parameters were analyzed according to KREBS (1978).

Materials and Methods

Fungal isolation

Collection of the plants from salt marsh at Delta, Manitoba (Canada), located at south end of lake Manitoba (50° II′ N, 98°

19′ W), were carried out at two-week intervals during the growing season of 1984. Samples were immediately processed for cauloplane and rhizoplane fungi. A total of seven collections, twenty plants of each species per collection, starting from June 6 and terminating September 6, were made. A total of 2520 root and shoot pieces from the six plants were plated out and examined. Roots and shoots of each plant species were cut into pieces of 2–4 mm length and washed thoroughly by using the washing technique described by HARLEY & WAID (1955) with some modifications: the washing, 20 changes, took place in a sartorius plastic filter apparatus as contamination was effectively reduced by aseptically adding and draining of water. The washed pieces were plated on Ohio Agricultural and Experimental Studies (OAES) medium (TUITE, 1969) and Malt extract medium, in lots of five pieces per plate to a total of 15 root fragments and 15 shoot fragments for each collection. Incubation was at 20±1° C, then the pieces were scored for growth of *Alternaria* species.

Data reduction and analysis

The overall total number of all species of *Alternaria* from the six plants was determined. Also the total number of isolates over the plants was used to calculate the percentage of isolations from each plant (number of isolates per plant divided by the total number of isolates from the six plants). The diversity was determined by dividing the number of species on each plant (S) by the total number of different species on all six plants (N). SIMPSON'S index (SIMPSON, 1949) was calculated as:

$$D_v = 1 - \Sigma[(p1)^2 + (p2)^2 + \ldots (pi)^n]$$

where D_v is the diversity index and p is the proportion of the number of isolates for each fungal species divided by the total number of isolates on each plant species (calculated from Table 1). Maximum diversity [MD = 1 − 1 / S] and percentage realized diversity [RD = D_v / MD × 100] was also determined. The percentage occurrence was determined by scoring the presence of each fungal species throughout the seven collecting dates on each plant species multiplied by one hundred. Frequency percentage of each fungal taxon was calculated by dividing the number of isolates of each species over the total number of isolates on each plant.

Dominance of *Alternaria* species on each plant was determined as following: the mean value ($\bar{X}$) and standard deviation (s) of isolates of all species on each plant was calculated. Fungal species with number of isolates more than $\bar{X}_1 + s_1$ were designated as dominant (D). A second mean value and standard deviation for all the species, ignoring the dominant species, were estimated. Fungi with number of isolates more than $\bar{X}_2 + s_2$ were considered subdominant (SD). Then a third mean value and standard deviation for the rest of fungi, ignoring the dominant and subdominant species, were calculated. Those fungi with number of isolates more than $\bar{X}_3 + s_3$ were called common (C). Fungi with number of isolates between the values of $\bar{X}_3 + s_3$ and $\bar{X}_3 - s_3$ were considered rare (R) while the species with number of isolates below the values of $\bar{X}_3 - s_3$ are called very rare (VR).

Results

12 taxa belonging to the genus *Alternaria* were recovered among 577 isolates from 2520 root and shoot pieces of the six plants (Table 1). Recovery of isolates on each plant is: on *Suaeda depressa* at 19.4%, *Salicornia europaea* at 18.6%, *Atriplex patula* at 16.3%, *Puccinellia nuttalliana* at 16.2%, *Hordeum jubatum* at 15.4%, and *Glaux maritima* at 14.1% (Fig. 1). Of 12 different *Alternaria* species, five represent slightly more than 90.0% of all the *Alternaria* isolates on the six plants. These include *A. alternata* (72.5%), *A. dennisii* (6.1%), *A. raphani* (4.2%), *A. chlamydospora* (3.5%), and *A. phragmospora* (3.0%). Other species (*A. citri, A. padawickii, A. petrosilini, A. pluriseptata, A. tenuissima, A. triticina* and *A. triticicola*) are all considered rare or very rare (Fig. 2). *Alternaria chlamydospora, A. petrosilini* and *A. phragmospora* are restricted to *Salicornia europaea, A. dennisii* to *Suaeda depressa,* and *A. padawickii* to *Glaux maritima,* while *A. triticina* and *A. triticicola* were found only on the graminoid *Hordeum jubatum* and *Puccinellia nuttalliana.*

SIMPSON's index ranges from 0.05 to 0.63 ($\bar{X}$ = 0.33±0.25) for *Alternaria* species on each plant for seven collections (Table 2). Diversity is low ($< \bar{X} - s$) on *Hordeum jubatum* and *Puccinellia nuttalliana* and it is high ($> \bar{X} + s$) on *Salicornia europaea* and *Suaeda depressa.* Maximum diversity (MD), on the other hand, does not generally change on these six plants while the realized diversity (RD) changes in relation to the changes of D_v (Table 2).

Temporal occurence of *Alternaria* spp. is shown in Fig. 3. *Alternaria padawickii* is the only species isolated during the early plant growth stages (from June 6 to June 23). Fungal species of the early to middle (June 6 to July 23) of the growing season are: *A. citri, A. phragmospora* and *A. pluriseptata.* Species appearing only late in the season (August 6 to September 6) include *A. triticina* and *A. triticicola,* while *A. chlamydospora* and *A. raphani* are recovered during the second half of season (Fig. 3). The most common species occurring across all the collecting period are *A. alternata, A. tenuissima* and *A. dennisii.*

Alternaria alternata was the most dominant species on all the six plants and over all collecting dates. This fungus constitutes

Table 1. Total number of isolates (T), Frequency (F), Occurrence percentage (OC) and Dominance function (DF) of twelve species of *Alternaria* recovered from six halophytes over the collecting period.

Fungal species	S. europaea				S. depressa				A. patula				G. maritima				H. jubatum				P. nuttalliana			
	T	F	OC	DF*	T	F	OC	DF	T	F	OC	DF	T	F	OC	DF	T	F	OC	DF	T	F	OC	DF
A. alternata (FR.) KEISSLER	49	45.8	95.7	D	58	51.8	100	D	71	75.5	85.7	D	68	84.0	100	D	84	94.4	100	D	88	93.6	100	D
A. chlamydospora MOUCHACCA	20	18.7	71.4	SD																				
A. citri ELLIS & PIERCE	3	2.8	14.3	VR	12	10.7	42.9	R																
A. dennisii ELLIS					35	31.3	85.7	SD																
A. padawickii (GANGULY) ELLIS													4	4.9	28.6	VR								
A. petrosilini (NEERG. ex SIMM.) ELLIS	11	10.3	28.6	R																				
A. phragmospora van EMDIN	17	15.9	57.2	C																				
A. pluriseptata (KARST. & HAR.) JORSTAD									4	4.3	28.0	R	9	11.1	28.6	R								
A. raphani GROVES & SKOLKO	7	6.5	42.9	VR					17	18.1	57.2	SD												
A. tenuissima (KUNZE: PERS.) WILTSHIRE					7	6.3	42.9	VR	2	2.1	14.2	VR												
A. triticina PARAS. & PROBHU																	3	3.4	14.3	R	2	2.1	14.3	VR
A. triticicola VASSAT PAO																	2	2.2	14.3	VR	4	4.3	28.6	R

S. europaea	S. depressa	A. patula	G. maritima	H. jubatum	P. nuttalliana
$\bar{X}_1 s_1 = 17.8 \pm 16.5$	$\bar{X}_1 s_1 = 28 \pm 23.4$	$\bar{X}_1 s_1 = 23.5 \pm 32.4$	$\bar{X}_1 s_1 = 27 \pm 35.6$	$\bar{X}_1 s_1 = 29.7 \pm 47.0$	$\bar{X}_1 s_1 = 31.3 \pm 49.1$
$\bar{X}_2 s_2 = 11.6 \pm 6.9$	$\bar{X}_2 s_2 = 18 \pm 14.9$	$\bar{X}_2 s_2 = 7.7 \pm 8.1$	$\bar{X}_2 s_2 = 6.5 \pm 3.5$	$\bar{X}_2 s_2 = 2.5 \pm 0.71$	$\bar{X}_2 s_2 = 3.0 \pm 1.4$
$\bar{X}_3 s_3 = 9.5 \pm 5.9$	$\bar{X}_3 s_3 = 9.5 \pm 7.5$	$\bar{X}_3 s_3 = 3.0 \pm 1.4$	$\bar{X}_3 s_3 = 6.5 \pm 0.0$	$\bar{X}_3 s_3 = 2.5 \pm 0.0$	$\bar{X}_3 s_3 = 3.0 \pm 1.4$

* D = Dominant, SD = Subdominant, C = Common, R = Rare, VR = Very rare

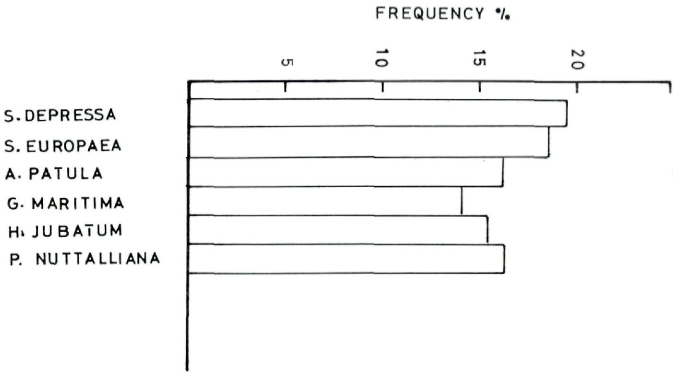

Fig. 1. Percentage frequency of *Alternaria* species on the six salt marsh plants over the collecting period.

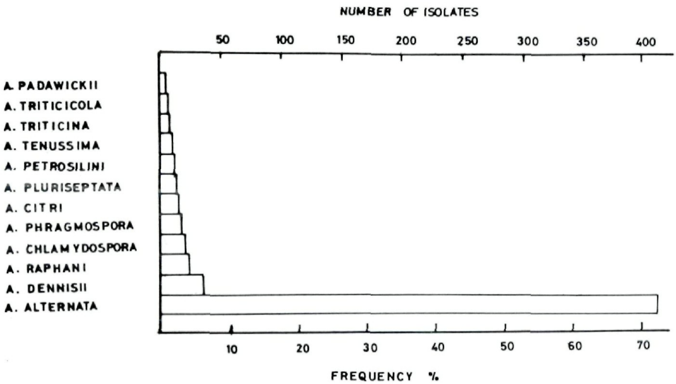

Fig. 2. Percentage frequency of isolations of twelve species of *Alternaria* on six salt marsh plants over the collecting period.

72.5% of the total *Alternaria* isolations (Table 1). Subdominant species include *A. chlamydospora*, *A. dennisii* and *A. raphani*. *Alternaria phragmospora* was the only fungus isolated as a common species on *Salicornia europaea*. The remnant species of *Alternaria* are rare or very rare (Table 1).

Table 2. Total number of species, total number of isolates, SIMPSON'S index, maximum diversity and realized diversity of species of *Alternaria* on each of the six halophytic plants.

Plant species	number of species	number of isolates	SIMPSON'S index (D_v)	maximum diversity	realized diversity
Atriplex patula	4	94	0.31	0.75	42%
Glaux maritima	3	81	0.27	0.67	40%
Hordeum jubatum	3	89	0.07	0.67	11%
Puccinellia nuttalliana	3	94	0.05	0.67	8%
Salicornia europaea	6	107	0.63	0.74	75%
Suaeda depressa	4	112	0.61	0.75	81%

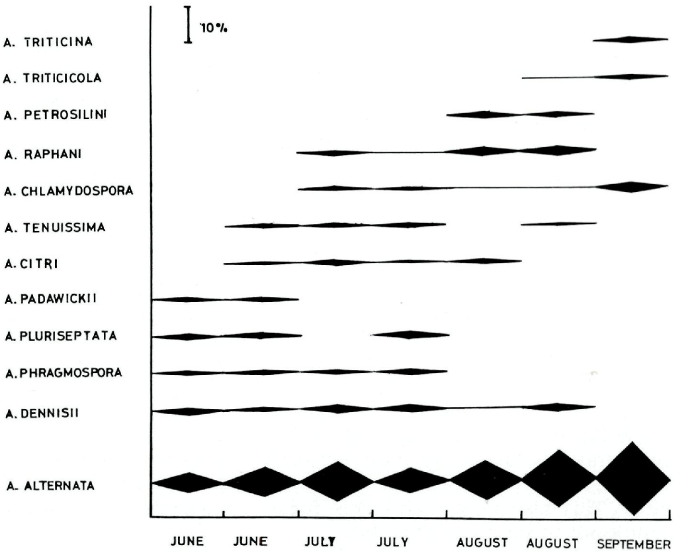

Fig. 3. Percentage occurrence and frequency of twelve species of *Alternaria* on six salt marsh plants on each collecting date. Vertical bar represents 10% frequency.

Discussion

An extensive survey of the genus *Alternaria* on six salt marsh halophytes revealed twelve different taxa. Their taxonomic position was described by MUSHIN & BOOTH (1987). Members of this genus were commonly observed on living and dead plants in salt marshes of Manitoba. On *Salicornia europaea* these fungi were more frequent than on the other plant species. The differences in the number of species and isolates recovered from the six halophytes may be attributed to the morphological and physiological nature of each plant. The surface-physical nature and chemistry of the plants, in general, are determining the presence or absence of fungi on plants (JOHNSON, 1975; CUTTER, 1976). Leachates produced by plants are also considered as a limiting factor (TUKEY, 1971). *Hordeum jubatum* and *Puccinellia nuttalliana* are non-succulent while *Salicornia europaea* and *Suaeda depressa* are succulent plants.

The differences in *Alternaria* species composition on the six plants are also possibly related to the osmotic strategies of these halophytes. *Salicornia europaea, Suaeda depressa* and *Atriplex patula* are considered obligate, while *Glaux maritima, Hordeum jubatum* and *Puccinellia nuttalliana* are facultative halophytes (CHAPMAN, 1974; UNGAR, 1974). Moreover, it has been documented that the salt level in tissues of *S. europaea, S. depressa* and *A. patula* is greater than in those of *G. maritima, H. jubatum* and *P. nuttalliana* (AUSTENFELD, 1974; ALBERT, 1975; FLOWERS, 1975; COOPER, 1982). Each of these plants, however, has a mechanism to control the salt level within its tissues (WAISEL, 1972). Concomitantly the associated *Alternaria* respond to the salt level in these plants (MUHSIN, 1985). No further studies are present to describe the relationship of mycoflora and salt marsh plants.

Alternaria alternata represents the dominant species and was isolated across the collecting period and associated with all the plants. Perhaps, the variety of exoenzymes produced by *A. alternata* (GESSNER, 1980) allows this organism to dominate and compete with other fungi on salt marsh plants. Furthermore, high conidial germination ratio of this fungus under high water moisture level (DICKINSON & BOTTOMLEY, 1980) may help the fungus to invade the substrate comparatively faster than other fungi. The ability of *A. alternata* to tolerate a wide range of salinity was shown (MUSHIN, unpublished) in vitro. This also may enable this fungus to survive under stress conditions in salt marsh habitats.

Dominance and diversity of *Alternaria* species are inversely related. When dominance is high (*Hordeum jubatum* and *Puccinellia nuttaliana*) the diversity is low. On the contrary, *Salicornia europaea* was colonized by six species of *Alternaria* (Table 1), i.e., high diversity correlates with a low number of isolates of the dominant species. These results are consistent with studies of other workers (SWIFT, 1976; FRANKLAND, 1981). High diversity of *Alternaria* species on *Salicornia europaea* probably can be attributed to the succulent nature of this plant.

Some of *Alternaria* species were found to be restricted to certain plant species. For example, *A. triticina* and *A. triticincola* were associated with the graminoid plants only, while *A. chlamydospora* and *A. phragmospora* were frequently sharing the same plant host (*Salicornia europaea*). More than one factor perhaps play a role in the selectivity of plant host by fungi in salt marshes. Salinity, however, seems the major limiting parameter under these conditions. Halophytes accumulate high amounts of osmotically active substances like amino acids; for instance non-succulent halophytes contain high levels of proline within their tissues. In comparison, succulent plants produce another type of amino acids, such as betain (STEWART & LEE, 1974; BRIENS & LARHER, 1982). Meantime some fungi, particularly *Alternaria* spp. were observed to produce high levels of L-proline (MISHRA & VYAS, 1972). Presumably the association of *Alternaria* species with stress-tolerant plants may be advantageous from the ecological point of view. Further research, however, is needed to elucidate the association of fungi and halophytes of salt marsh habitats.

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References

ALBERT, R. (1975). Salt regulation in halophytes. – Oecologia 21: 757–771.

APINIS, A. E. & C. G. C. CHESTERS (1964). Ascomycetes of some salt marshes and sand dunes. – Trans. Brit. Mycol. Soc. 47: 419–435.

AUSTENFELD, F. A. (1974). Correlation of substrate salinity and ion concentration in *Salicornia europaea* L. with special reference to oxalate. – Biochemie & Physiol. Pflanzen 165: 303–316.

BRIENS, M. & F. LARHER (1982). Osmoregulation in halophytic higher plants: a comparative study of soluble carbohydrates, polyols, betains and free proline. – Plant Cell and Environ. 5: 287–292.

CHAPMAN, V. J. (1974). Salt marshes and deserts of the world. – In: Ecology of Halophytes. Edited by R. J. REIMOLD & W. H. QUEEN, Academic Press, New York, pp. 3–19.

COOPER, A. (1982). The effects of salinity and waterlogging on the growth and cation uptake of salt marsh plants. – New Phytol. 90: 263–275.

CUTTER, E. G. (1976). Aspects of the structure and development of the aerial surface of higher plants. – In: Microbiology of Aerial Plants Surfaces. Edited by C. H. DICKINSON & T. F. PREECE, Academic Press, New York, pp. 1–40.

DICKINSON, C. H. (1965). The mycoflora associated with *Halimione portulacoides*. III. Fungi on green and moribund leaves. – Trans. Brit. Mycol. Soc. 48: 603–610.

— & D. BOTTOMLEY (1980). Germination and growth of *Alternaria* and *Clados-*

porium in relation to their activity in the phylloplane. – Trans. Brit. Mycol. Soc. 74: 309–319.

FLOWERS, T. J. (1975). Halophytes. – In: Ion Transport in Plant Cells and Tissues. Edited by D. A. BAKER & J. L. HALL, North Holland Public. Co., pp. 309–334.

FRANKLAND, J. C. (1981). Mechanism in fungal community: Its organization and role in the ecosystem. – In: The fungal community. Edited by D. T. WICKLOW & G. C. CARROLL, Marcell Dekker Inc., New York, pp. 403–426.

GESSNER, R. V. (1977). Seasonal occurrence and distribution of fungi associated with *Spartina alterniflora* from a Rhode Island estuary. – Mycologia 69: 477–491.

— (1980). Degradative enzyme production by salt marsh fungi. – Botanica Marina 23: 133–139.

HARLEY, J. L. & J. S. WAID (1955). A method of studying active mycelia on living roots and other surfaces in the soil. – Trans. Brit. Mycol. Soc. 38: 104–118.

HENDERARTO, I. B. & C. H. DICKINSON (1984). Soil and root microorganisms in four salt marsh communities. – Trans. Brit. Mycol. Soc. 83: 615–620.

JOHNSON, H. B. (1975). Plant Pubescent. An ecological perspective. – Bot. Gaz. 41: 233–258.

KOHLMEYER, J. & E. KOHLMEYER (1979). Marine Mycology: The higher fungi. – Academic Press, New York, 690 pp.

KREBS, C. J. (1978). Ecology: The experimental analysis of distribution and abundance. – Harper and Row Publs., New York, 678 pp.

MISHRA, A. S. & K. M. VYAS (1972). Production of L-proline by soil microorganisms. – Hindus. Antibot. Bull. 15: 30–32.

MUHSIN, T. M. (1985). Studies on fungi associated with halophytes from Delta Marsh, Manitoba. – PhD Thesis, University of Manitoba, Canada, 318 pp.

— & T. BOOTH (1987). Fungi associated with halophytes in an inland salt marsh, Manitoba, Canada. – Can. J. Bot. 65: 1137–1151.

PUGH, G. J. F. (1974). Fungi in intertidal regions. – Veröff. Inst. Meeresforsch. 5: 403–418.

SIMPSON, E. H. (1949). Measurement of diversity. – Nature 163: 688.

STEWART, G. R. & J. A. LEE (1974). The rôle of proline accumulation in halophytes. – Planta 120: 279–289.

SWIFT, W. J. (1976). Species diversity and the structure of microbial communities in terrestrial habitats. – In: The rôle of terrestrial and aquatic organisms in decomposition processes. Edited by J. M. ANDERSON & A. MACFADYAN, Blackwell Scientific Publ., England, pp. 185–222.

TUITE, J. (1969). Plant pathological methods. – Burgess, Minneapolis, 239 pp.

TUKEY, H. B. (1971). Leaching of substances from plants. – In: Ecology of leaf surface microorganisms. Edited by T. F. PREECE & C. H. DICKINSON, Academic Press, New York, pp. 67–80.

UNGAR, I. A. (1974). Inland halophytes of the United States. – In: Ecology of halophytes. Edited by R. J. REIMOLD & W. H. QUEEN, Academic Press, New York, pp. 235–305.

WAISEL, Y. (1972). Biology of halophytes. – Academic Press, London, 395 pp.