UNIVERSITY OF SOUTHAMPTON

I give here my consent that my thesis entitled An investigation of "die-back" in Spartina townsendii H. & J. Groves upon which the degree of Ph.D. was awarded to me by the University of Southampton in March 1957 may be made available for inter-library loan or for photo-copying.

Signed [redacted]

Date 27th March 1957.

AN INVESTIGATION OF "DIE-BACK" IN SPARTINA TOWNSENDII H. & J. GROVES.

A Thesis submitted by

Peter J. Goodman

For the degree of Doctor of Philosophy

in the University of Southampton.

January, 1957.

Botany Department,
The University of
Southampton.

ABSTRACT.

An investigation of "die-back" in Spartina townsendii

H. & J. Groves.

The nature, extent, and causes of "die-back" in Spartina townsendii have been investigated. The "die-back" areas are fringed by dying plants, whose rhizomes are soft rotted, without associated primary pathogens. Such plants recovered when brought into laboratory sand culture, and produced healthy rhizomes.

An ecological survey revealed "die-back" extensively on the south coast; in one area on the east coast; and not at all on the west coast. It is found at relatively low levels both beside main channels and in "pans" in Spartineta.

The growth of S. townsendii has been studied in culture, and failures in tillering and rhizoming have been traced in the field. Rhizomes and tillers failed in offsets transplanted into "die-back" areas.

Healthy and dying plants showed slight differences in their contents of the major mineral elements, but these were not reflected in analyses of the soils.

Soil solutions from "die-back" areas supported germination of seeds; but S. townsendii offsets, in

laboratory conditions, grew badly in untreated soil.

"Die-back" soils were found to have exceptionally high contents of water and organic matter, and to be fine particled, charged with sulphides, and strongly reducing, causing anaerobic respiration of rhizomes. Anaerobic, sulphide containing, laboratory cultures have been made, to reproduce the conditions of "die-back".

"Die-back" is considered to be caused by the toxic effects of sulphides produced by bacterial action in the waterlogged substrates which result from accretion by Spartina of fine particled silts.

CONTENTS.

APPENDICES.

INDEX TO TABLES.

Appendix 5.

INDEX TO FIGURES.

Appendix 4.

An investigation of "die-back" in Spartina townsendii

H. & J. Groves.

I. INTRODUCTION.

Spartina townsendii[1] was collected near Hythe, Southampton Water, in 1878, by the brothers Groves, who described it shortly afterwards as a new species (Groves, H. & J., 1881). Subsequent investigation by Sutherland and Eastwood (1916) revealed that earlier collections had been made, in 1870, and that there had possibly been even earlier ones than that.

From Hythe the grass spread along the coast to east and west. By 1915 it was established in every estuary and salt marsh from Chichester to Poole, and in the Isle of

---

[1] Recent investigations by Hubbard (unpublished) have revealed that the earliest specimens were of a more slender male sterile grass, but that later collections were principally of a vigorous fertile grass. The present investigation is concerned with swards of mainly fertile plants, but including rare male sterile plants.

The suggestion has been made that the fertile form, with a chromosome number of (2n = 126) is a different species from the male sterile form, which is still found locally on the south coast, with a chromosome number of (2n = 63).

In this work, when specific reference is made to male sterile plants, they are called Spartina townsendii^1. When specific reference is made to fertile plants, they are called S. townsendii^2. No number is added to the binomial when the sward of mainly fertile plants, with rare male sterile plants, is mentioned.

Wight (Stapf, O., 1908; Oliver, F.W., 1920 and 1925). Spartina occupied the level immediately above that occupied by the Zostera spp. of these areas (Oliver, F.W., 1925). In 1918 - 1933 the inadequately explained Zostera "disease" (Butcher, R.W., 1935) left Spartina as the only phanerogam on an area estimated from aerial survey photographs to be 9,000 acres. As Spartina colonized this area, accretion increased greatly, and the whole regime of siltation changed (Oliver, F.W., 1920).

After 1910 Spartina was planted extensively in Britain, often by private land owners for reclamation work (Oliver, F.W., 1920; Roper, I., 1922; Bryce, J., 1931; Yapp, R.H., 1923; and personal communications). It is now found in England, Scotland, Wales, and Ireland, covering at least 30,000 acres of mud flats.

Spartina townsendii has also spread or been planted in Europe from Denmark to Brittany (Jørgensen, C.A., 1934; Jacquet, J., 1949; Konig, D., 1949), and in other parts of the world (Bryce, J., 1941), notably with success in New Zealand (Allan, H., 1931). Altogether the grass now covers hundred of square miles, and has a wide distribution in temperate latitudes.

Reports of patchy degeneration of previously healthy sward - "die-back" - came first from Beaulieu, in 1930 (Mills, D. in litt.). Other reports followed, from Lymington, in 1939 (Cooper, S., verbal communication), and from Keyhaven (Austwick, P.K.C., 1950). "Die-back" has also been seen by the present author at Havant, in 1950; Poole, in 1954; Portchester, in 1955; and reported from West Wittering in 1955 (Buckle, O., verbal communication). According to recent surveys, altogether some 150 acres of Spartinetum have been lost and 1,500 acres are affected. Only one of the younger, planted areas outside the Hampshire Basin, the Essex Stour, shows "die-back". No "die-back" has been reported by recent workers on the Continent (Jacquet, J., 1949), though Jørgensen (in litt., 1953) reports "stunting due to soil conditions" in Denmark. No death occurs, and this is apparently an effect distinct from "die-back" as it occurs in England.

Of all the "die-back" areas, the most seriously affected is Lymington. The area of bare mud, once Spartinetum, now amounts to 90 acres. Recent surveys have shown that almost the whole sward of over 500 acres is affected by "die-back" to some extent. Recession of the grass from the main river channel was first noticed by local residents in 1939, and was associated with the apparently large dredging requirement of the river from 1946 - 1949 by the dredging authority, British Railways, who run a ferry service from Lymington to Yarmouth, Isle of Wight (Jellett, J. in litt., 1949): (Table I).

TABLE I.

Dredging Requirements at Lymington and Newhaven, 1946 - 1949.

Port.	Quantity dredged 1946-1949 cu.yds. in situ.	Area maintained in acres.	Catchment area sq. miles.	Quantity dredged/ acre/sq. mile.
Lymington	81,000	23·4	8·79	394
Newhaven	600,000	56·9	47·59	221

In the hope of checking "die-back" and lessening the cost of dredging, the Railway Authorities sought the help of the Director of the Royal Botanic Gardens, who sent first Mr. C. E. Hubbard, then Mr. P.K.C. Austwick, to report on the "die-back" (Austwick, P.K.C., 1950).

Austwick (1950) followed a single field excursion with a six month examination of Spartina material. He listed 20 possibly pathogenic fungi, and sought, unsuccessfully, other pathogens. He also reviewed the physical and dynamic factors of the habitat. He considered the problem to be complex, and recommended further search for pathogens, a wide ecological survey, transplanting experiments, and an investigation of the physical and chemical nature of the substrate.

The present investigation was begun in July 1953 under a grant from the Agricultural Research Council with the object of extending the observations made by Austwick (1950), surveying the scope of the problem, and, if possible, finding the cause of "die-back", and whether it could be countered.

Based on the suggestions of Austwick (1950), and as the work developed, the following lines of investigation were pursued:-

1. An investigation was made into the possibility of fungus or other pathogen attack, and those organisms found were tested for pathogenicity.
2. A survey of Spartina was made throughout the British Isles, so that its behaviour locally could be appreciated. The later stages of this survey were carried out in collaboration with Miss E.M. Uren, who began work on the more ecological aspects of the problem in July 1955, and some reference is made to certain of her unpublished data.
3. The growth and morphological behaviour of the plant were investigated under different conditions.
4. Physical and chemical conditions of the substrate were analysed, and experimental cultures made, to test the effect of substrate conditions on the plant.

Contributions to a Biological Flora account of the species are added as an appendix.

II. INVESTIGATION OF THE POSSIBILITY OF PATHOGEN ATTACK.

A. Previous work.

Austwick (1950), as a result of his brief survey, considered attack by pathogens other than fungi unlikely. Previously, Spartina townsendii had only two associated fungi on record, Didymosphaeria spartinae Grove, and Hendersonia culmicola Sacc., both found by Grove (1933, 1937) In Poole Harbour.

In related Spartina species, however, the following important fungi were recorded:-

Leptosphaeria spp. (Ellis, J.B. & Everhart, B.M., 1885).
Halophiobolus medusa (Barghoorn, E.S. & Linder, D.H., 1944).
Uredo peridermiospora (Ellis, J.B. & Tracy, S.M., 1890), together with species of Uromyces, Phyllosticta, and Fusarium (Saccardo, P.A., 1898).

The first collection of fungi extensively from healthy S. townsendii sward was made by Lloyd (1952). No living plants examined bore fungi. Dead and decaying stems and leaf sheaths, however, often bore mycelium and fruit bodies of fungi. Pycnidia and perithecia were found occupying the air chambers of the stems. Stems on the drift line bore apothecia. Seven fungal species were collected and identified provisionally as follows:-

? Scleroplea sp. On leaf sheaths.

? Massarina sp. On dead and decaying leaf sheaths.

Halophiobolus medusa ? On dead stems.

? Patinella sp.)
) On detached stems on the drift line.
? Helvella sp.)

Stagonospora sp. On dead and decaying leaf sheaths and inflorescences.

Phoma sp. On dead leaf sheaths.

Cladosporium herbarum has also been collected in this area (Chater, E.H. in Lloyd, L.S., 1952).

The first collection of fungi from Spartina sward where "die-back" was occurring was made by Austwick (1950). He reported that no living plants examined from the edges of "die-back" areas at Lymington bore any distinct fungal parasite, nor had they any single specific symptom.

All the fungi collected by Austwick (1950) have been collected again by the present author, with the following exceptions which were collected by Austwick only:-

Phytophthora sp.)
) On excised leaves in culture.
Pythiogeton sp.)

Chaetomium cochloides Palliser. In one moist culture of dead leaves.

Epicoccum purpurascens Ehrbg. On damaged spikes.

Volutella ciliata (Alb. & Schw.) Fr. In a moist culture of rhizomes.

Austwick has since remarked (verbal communication) that the Phytophthora and Pythiogeton spp. may have developed on some algae associated with the excised leaves.

B. Examination of Material, 1953 - 1956.

Further search was made between 1953 and 1956 for possible pathogens on plants at the edges of the "die-back" areas at Lymington and elsewhere. Regular two-monthly collections were made at Lymington between August 1953 and August 1954, to detect any possible seasonal attack.

1. Symptoms of "die-back".

Living material was rarely infected by other than one fungus - ? Ligniera sp. The leaves of living plants were sometimes torn from their sheaths at the pulvinar articulation by the force of the tide, however, and fungal mycelium was then occasionally found near the point of damage. This damage was common in plants from the sward on the east of the estuary mouth at Lymington.

Living plants on the "die-back" edges that were apparently dying were yellowish, with rather few tillers, so that the proportion of younger parts was decreased, and the plants became moribund. Tillers and rhizomes were weak.

Failure of the rhizomes was the most characteristic symptom of "die-back". Healthy rhizomes were strong and turgid, and sections stained with iodine showed abundant starch. "Die-back" rhizomes were weak and flaccid, with little starch. The air chambers were large, and the tissue around them was soft. The weakest area was that just behind the vegetative apex, where the air chambers begin to form.

Material examined in a state of weakness had no associated fungi or bacteria. As the state of unhealthiness progressed, the rhizomes, and the roots as well, became "soft rotted", all the tissue but the tougher elements being destroyed. Apparently secondary bacterial infection occurred at this stage. In one case a barrier layer was seen, showing that infection had occurred in a rhizome still just alive, but this was exceptional.

The secondary infection, it seemed, did no more than achieve the final rotting of the tissue, leaving only the vascular and cortical elements, together with the remains of the scale leaves (Fig. 1).

There was no sign of any general primary pathogen attack, either by fungi or other organisms.

2. <u>Fungi isolated</u>.

Dead material consisted of the lowest nodes of the stem, the air passages of which contained perithecia and pycnidia, and the "soft rotted" roots and rhizomes. The following fungi were isolated from this material:-

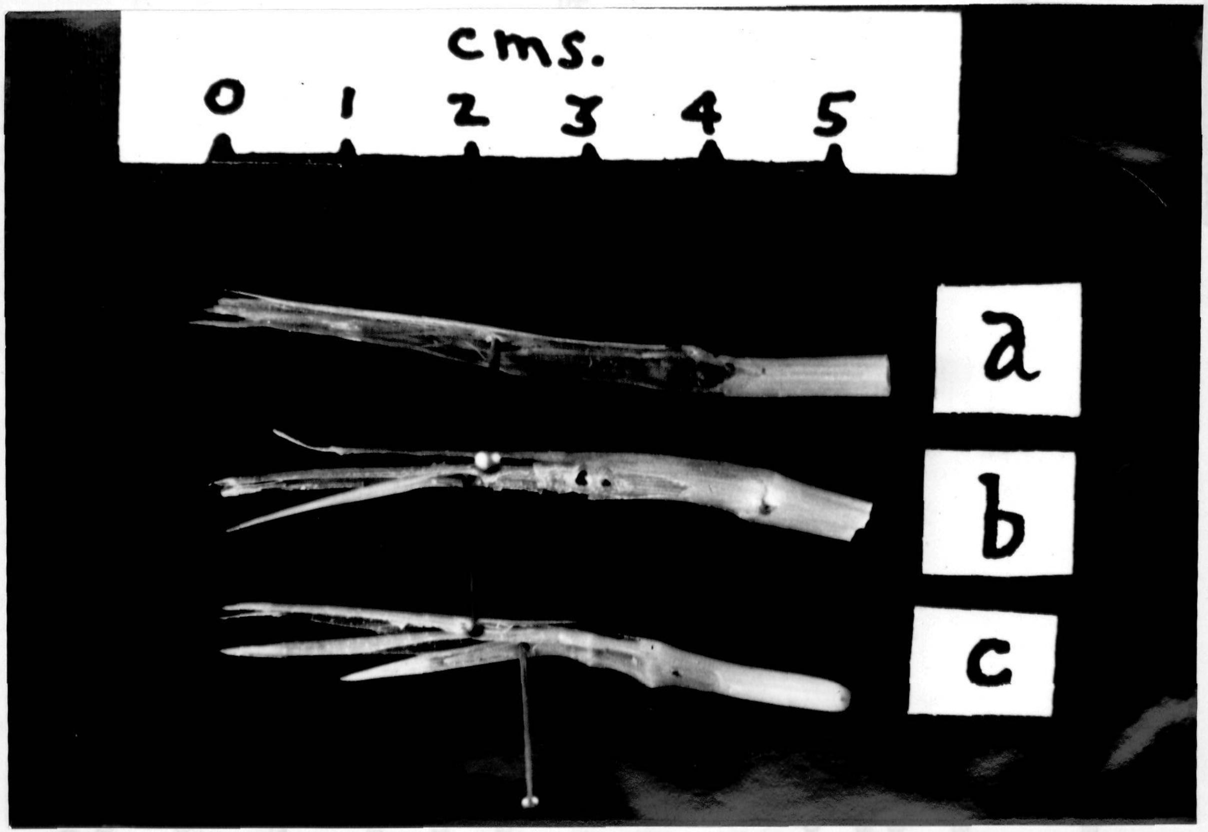


FIG. I. "Soft rotted" rhizomes from the Spartina sward near Lymington Pier.

(a) Apex completely disintegrated - sheathing leaves only, remaining.
(b) Feeble apex remaining, with point of weakness behind.
(c) Vigorous healthy apex with strong sheathing leaves.

Lymington 1953 - 1956.

Phycomycetes.

? Ligniera junci (Schw) Maire & Tyson   In living roots.

Rhizopus nigricans Ehrenberg.   On old stems.

Ascomycetes.

Didymosphaeria spartinae Grove.   On old stems.

Amphisphaeria culmicola Sacc.   On old stems, lower river only, abundant February.

Leptosphaeria typharum (Desm) Karst.   On old stems.

Pleospora salsolae Fuckel.   On old glumes and rhaches, abundant November.

Halophiobolus medusa (Ell. & Ev.) Linder.   On oldest parts, upper river only.

Fungi imperfecti.

Cladosporium herbarum Link ex Fries.   On old stems.

Botrytis cinerea Pers. ex Fr.   On old stems.

Alternaria maritima Sutherland.   On old stems of various ages.

Penicillium spp.)
) On old parts, fairly frequently.
Aspergillus spp.)

Phoma sp. Surface mycelium commonly present all the year round.

Stagonospora sp.   On old leaves and stems abundantly all the year round.

Cephalosporium sp.   On one culture of old leaves.

Poecilomyces sp.   On one culture from "die-back" in sward.

Acrothecium sp.   On one culture of roots.

Coniothyrium sp.   On one culture from "die-back" in sward.

In addition to the fungi isolated at Lymington, the following fungi have been collected from other Spartineta:-

Phycomycetes.

?Ligniera junci In living roots, healthy Spartinetum, Essex.

Ascomycetes.

Halophiobolus medusa. On oldest parts, "die-back" areas, Poole, Havant, Portchester.

Leptosphaeria typharum. On old stems, "die-back" areas, Poole, Havant.

Fungi imperfecti.

Phoma sp. Surface mycelium, "die-back" areas, Eling, Havant, Poole.

All except Ligniera were isolated and grown in culture either from single asci or spores removed from the surface sterilized fruit bodies, or from surface sterilized Spartina material.

A number of cultures remained sterile and were therefore unidentifiable. In the more difficult groups, critical identifications were not always possible, but whenever practicable the determinations were confirmed by specialists.

3. Discussion.

None of the fungi collected can certainly be identified as an obligate parasite. None of the rusts reported on other Spartina species has been found on S. townsendii. The likely parasites Phytophthora and Pythiogeton have not appeared in surface sterilized material in later collections than that of Austwick (1950). This suggests that they were, in fact, growing on algae associated with the Spartina leaves in Austwick's culture.

Two fungi collected are noteworthy as being possible facultative parasites. These are:-

a. ? Ligniera junci.
b. Halophiobolus medusa.

Species of Stagonospora and Phoma are also known to be parasitic.

a. ? Ligniera junci.

This fungus alone was common in living material. It occurred near the root tips in the Spartina at Lymington, and in one healthy site in Essex. No signs of hypertrophy have been found in connection with its presence. Absence of hypertrophy is characteristic of the genus (Cook, W.R.I., 1926). The spore size of the collected material differs slightly from the type. Without full knowledge of the life history, it cannot certainly be identified. It is a possible, though unlikely, parasite.

b. Halophiobolus medusa.

A short spored Halophiobolus sp. was associated with the decline of Zostera marina in Canada (Mounce, I. & Diehl, W.M., 1934) and examination of type specimens has shown that the two, though distinct, are related (Wilson, I. unpublished). This relationship makes H. medusa a suspect parasite, though other members of the genus are saprophytic. At Lymington it is found in the upper river only, a distribution more restricted than that of "die-back".

Other than the species of Stagonospora and Phoma, the remaining fungi were almost certainly saprophytic. The distribution of the fungi Halophiobolus in the upper river and Amphisphaeria in the lower river suggested that they were marine saprophytes distributed according to some estuarine ecological factor.

While the fungal flora was predominantly saprophytic, there still remained unidentified mycelia, and suspect species such as those of Stagonospora and Phoma, whose status could only be determined by infection experiments. These were accordingly set up.

C. Infection experiments.

Austwick (1950) suggested that infection experiments should be carried out to determine whether the fungi collected in "die-back" areas were pathogenic. This was especially needed in the case of fungi which could not be identified with certainty.

The first experiments were carried out in the laboratory in case any infection might spread. Later, tests in the field were planned by transplanting unhealthy material into healthy sward.

The first experiments were merely attempts to pass the infection from unhealthy material from the edges of "die-back" sites, to material from healthy sward.

1. Plant - plant infection.

Heavily fungus infected part living plants were brought in from three Lymington "die-back" sites and set in 9" pots of garden soil beside plants from healthy Spartinetum whose roots had first been thoroughly washed. Three controls were set up, with no infected plant added. Each plant stood in a saucer of fresh water.

After a year, the condition of the plants was examined (Table II).

TABLE II.

Lymington site.	Organisms present. September 1953.	One year state of plants. September 1954.	
		Lymington plant	Whippingham plant
King's Post. MR 33189535 [x]	*Halophiobolus* *Leptosphaeria* *Stagonospora* *Pleospora*	Alive	Dead
Tar Barrel. MR 34139433	*Stagonospora* *Didymosphaeria* *Phoma* & Brown mycelium?	Dead	Alive
Eastlake. MR 34489405	*Didymosphaeria* *Alternaria* *Leptosphaeria* *Amphisphaeria* Brown mycelium?	Alive	Dead
Control No.1	No Lymington plant	------	Alive
Control No.2	No Lymington plant	------	Alive
Control No.3	No Lymington plant	------	Dead

[x] Map References on 6" Provisional Edition & Air Survey Map (below).

The experiment was invalidated by the death among the controls, but the condition of the unhealthy plants from the "die-back" edges was remarkable. Those which survived grew on well (Fig. 2) until they were as healthy in appearance as the original healthy plants.

2. Seedling-spore infection.

Following the unsuccessful attempt to infect *Spartina* plants, an attempt was made to infect seedlings, as it was thought these might be more susceptible to infection.

Chopped *Spartina* material containing *Ligniera* was added to pots of sand cultured *Spartina* seedlings, and "uninfected" controls were set up. No deaths occurred in either the "infected" or the "control" experiments.

Repetition of the experiment with chopped material containing *Amphisphaeria*, *Didymosphaeria*, and other unidentified fungi also failed to produce any death or symptoms.

3. Seedling - plant infection.

Introduction of infected *Spartina* plants, with fungi on their dead parts, was next attempted in seedling cultures in sand. The pots were watered with culture solution.

After a few weeks the seedlings were still strong and healthy, with no deaths or symptoms. What was

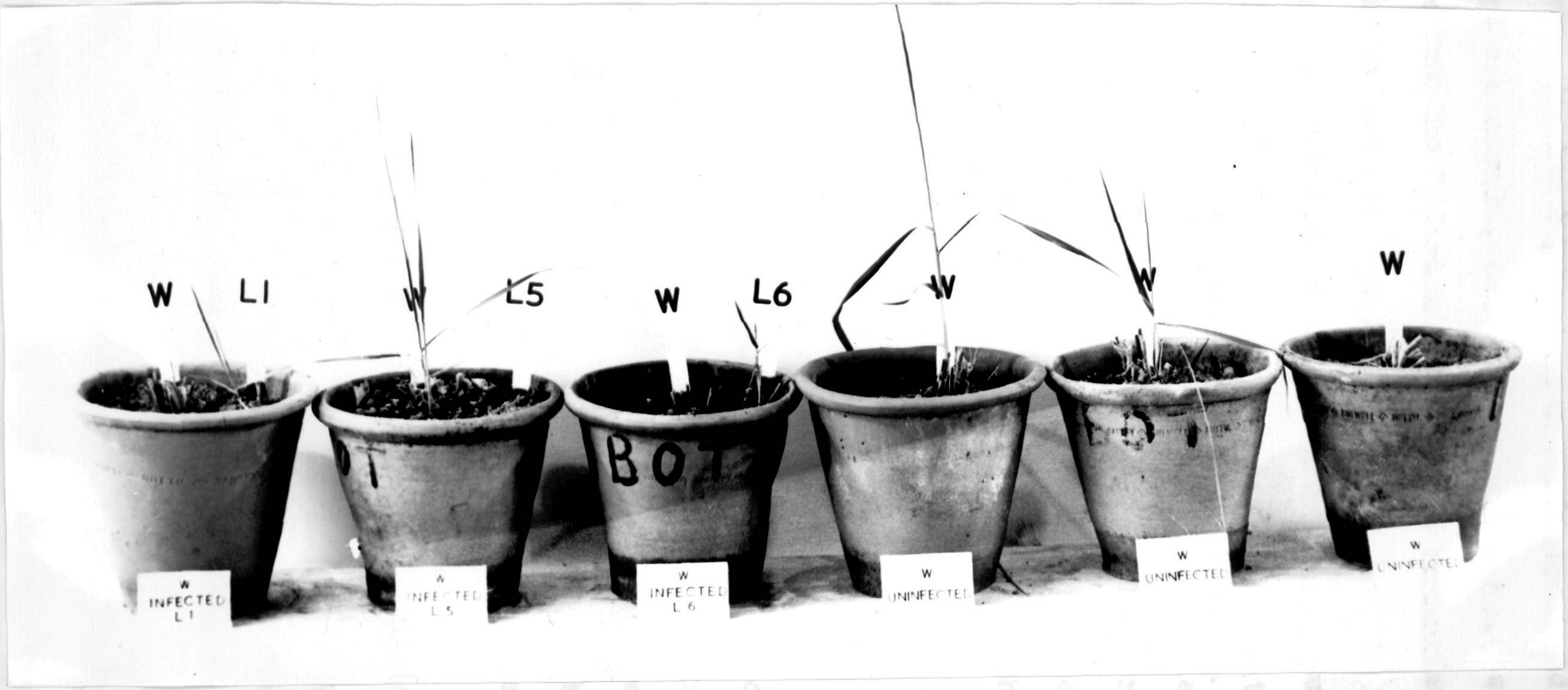


FIG. 2. Infection experiment: heavily fungus infected Lymington plants (L) versus healthy Whippingham plants (W); after one year. With uninfected controls.

L_1 = King's Post, MR 33189535.
L_5 = Tar Barrel, MR 34139433.
L_6 = Eastlake, MR 34489405.

Note "recovery" of L_1 and L_6 and occasion deaths of W plants.

remarkable, however, was again the condition of the unhealthy plants. These showed distinct signs of recovery. This continued until the old unhealthy parts of the plants were hidden in the new flush of growth. They continued to grow rapidly, and tillered well, eventually flowering. This experimental "recovery" was repeated several times.

By this time it was apparent that infection could not be passed from one plant to another in laboratory conditions, each time this was attempted, the plants replacing the old, unhealthy parts with new and vigorous parts. This phenomenon was further investigated.

D. Recovery experiments.

Further investigation was made of experimental "recovery" of plants from "die-back" areas. Part living plants with fungi on their dead parts were brought in from five areas on the edge of the "die-back" zones along the main channel side at Lymington. The plants looked very unhealthy (Fig. 3).

After a year's culture in sand, with mineral nutrients supplied in solution, the plants were quite healthy and flowering (Fig. 4). The changes that had occurred during this time are set out in Table III.

A second batch of plants was taken from the "die-back" areas in the sward at Lymington. These also looked very unhealthy. The first batch from the in sward "die-back"

FIG. 3. Unhealthy plants from Lymington "die-back" sites, September 1954.
L_1 = King's Post. L_3 = Pier. L_4 = No. 11 Post.
L_5 = Tar Barrel. L_6 = Eastlake. (see Table III).
Note amount of moribund material in the clumps.

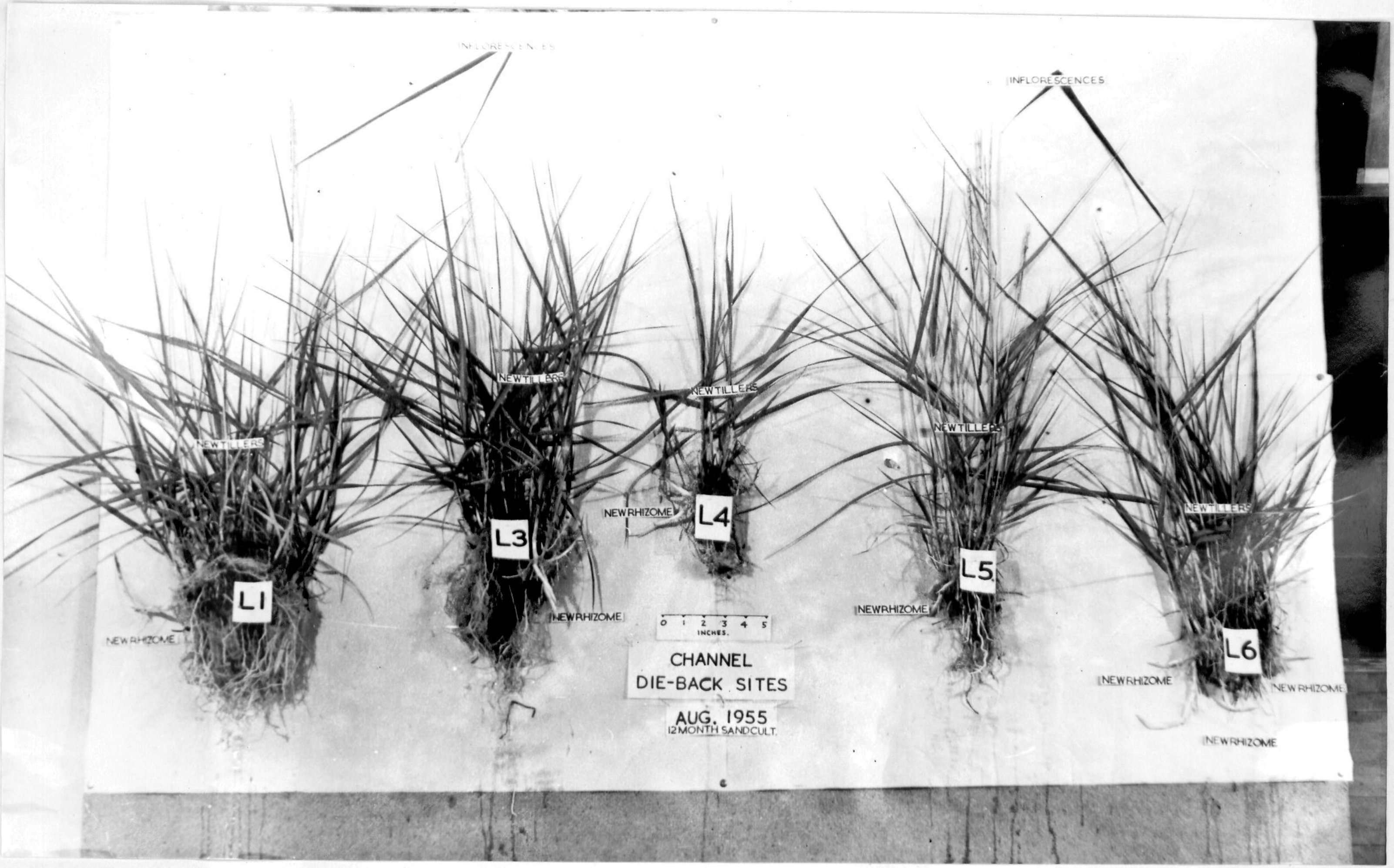


FIG. 4. The same plants as in Fig. 3 after a year's sand culture. Note the strong healthy growth and formation of new inflorescences. (see Table III).

TABLE III.

Plant Recovery from Infection.

Lymington site.	Beginning of experiment.	End of experiment.
King's Post (L1) MR 33189535	11 shoots.	26 shoots. 2 inflorescences. 12 rhizomes
Pier (L3) MR 33409535	13 shoots.	28 shoots 1 inflorescence 7 rhizomes
No.11 Post (L4) MR 33709493	5 shoots.	7 shoots 0 inflorescences 4 rhizomes
Tar Barrel (L5) MR 34139433	7 shoots.	24 shoots 6 inflorescences 4 rhizomes
Eastlake (L6) MR 34489405	13 shoots.	32 shoots 4 inflorescences 8 rhizomes

areas was killed by drought resulting from the severe frosts in early 1956, when plants in the Spartineta also suffered damage. Another batch brought in to repeat the experiment recovered quite normally, producing tall vigorous plants.

It was evident from these further trials that recovery of unhealthy plants brought in from the edges of "die-back" sites was a general phenomenon.

E. Transplanting experiments.

Transfer of plants from the field to the laboratory had already shown interesting results. It was decided to set up transplanting experiments in the field.

Transplanting experiments had been suggested by

Austwick (1950) as a means of testing the suitability of the "die-back" areas for supporting Spartina growth. A large experiment was carried out at Lymington in 1953, and further ones have been carried out there and elsewhere, on a smaller scale, in subsequent years.

The method of taking cuttings described by Carey and Oliver (1918) was tested in an area of Poole Harbour cleared of Spartina by a freak storm in November 1954. Twelve plants set in the denuded area grew strongly, and were flowering within a year of setting, initiating strong tussocks.

1,250 strong cuttings were taken from the healthy sward at Sandbanks, as in the test, and set in the Lymington channel "die-back" zone within a few days of collection. A year later, their growth was recorded (Table IV).

The great majority of plants failed. The difference in "take" between the upper and lower river sites appears significant, only 2 out of 875 plants in the upper four sites having grown on, while 11 out of 375 grew on in the lower two sites, even though 60 plants are known to have been washed away from one of them (the Eastlake site), in the November 1954 gales. The remainder of the planted area was lost in the first six months of 1956.

The symptoms attending failure differed in the upper and lower river sites. In the upper river sites the plants died quickly, like those in "die-back" conditions,

TABLE IV.
Results of Transplanting Experiments.

Planting site.	Area planted (sq.yds)	No. of living plants.		Tillers per plant.	Rhizomes per plant.	Remarks
		Dec.'53	Nov.'54.			
Walhampton MR 33059558	100	125	0	-	-	Very little erosion 100% die-back.
King's Post MR 33189535	200	250	0	-	-	" "
Pier MR 33409535	200	250	2	1, 1.	-	Very little erosion 99% die-back.
No. 11 Post MR 33709493	200	250	0	-	-	Slight erosion 100% die-back.
Tar Barrel MR 34139433	200	250	8	3,2,2,2, 2,2,2,1	2, 1.	All damaged; much erosion.
Eastlake MR 34489405	100	125	3	1,5,3	1, 1.	All damaged; strong erosion ½ site washed away.

with no definite symptoms save the failure of rhizome and tiller production.

By contrast, the plants set in the lower sites, where erosion by wave action is apparently occurring, first became dwarfed, with torn leaves, like the plants around them. They finally died, though rather slowly, cuttings evidently not being able to get established under these conditions.

Other experiments in the Lymington area, in which healthy plants were set in "die-back" areas in the sward, resulted in the death of the majority of the plants within a year. Similar small scale trials at Havant and Beaulieu resulted in all the plants dying within a year.

Experiments in which plants were transferred from the edges of both the in sward "die-back" and that beside the main channel to healthy sward resulted in a high "take", from 6 - 11 plants out of 12 set in each experiment, recovering. These plants grew on successfully, becoming tall and healthy, in the same way as the plants brought in from the "die-back" sites to laboratory sand culture.

These experiments showed conclusively that "die-back" was associated with definite sites, in which introduced healthy plants died. By contrast, unhealthy plants from the edges of "die-back" sites recovered when removed from them.

F. Conclusions.

"Die-back" plants were found to have a generally unhealthy appearance, being yellowed and failing to grow. The few tillers produced were weak, and the rhizomes and roots soft rotted.

No obvious primary pathogens were found, and, though facultative parasites may have been present, these were not strong enough to attack the plant in laboratory culture.

Unhealthy plants from the edges of "die-back" sites, both beside the main channel and in the sward, "recovered", growing and flowering, when transferred to laboratory sand culture, and also when transferred to healthy sward.

Healthy plants set in the "die-back" zones at Lymington, both beside the main channel and in the sward, died. There was swifter acting "die-back" in the upper river at Lymington than in the lower river.

Plants thus showed a very marked "site effect", dying in unhealthy sites, recovering in healthy sites. This emphasised the need for an ecological survey of the habitats in which the plant grew. There was also the need for more accurate methods of assessing the behaviour of the grass. Ecological and morphological studies were begun.

III A. GENERAL SURVEY.

On the suggestion of Austwick (1950) a general ecological survey of Spartina townsendii was undertaken, commencing in 1953 with primary studies on the south coast, the Stour (east coast), and the Dovey (west coast). The east and west coasts have since been surveyed more widely in collaboration with Miss E. M. Uren, who began work on the detailed ecological aspects of the problem in July 1955.

Ground survey of Spartineta was assisted by the use of unpublished ground photographs taken in the past by Professors F.W. Oliver, S. Mangham, and others. Aerial oblique photographs have also been obtained from Aerofilms Ltd., and Dr. J.K. St. Joseph, Curator in Aerial Photography in the University of Cambridge. Mapping of Spartineta and "die-back" areas has been possible by use of vertical air mosaic photographs and stereoscopic pairs. Almost complete surveys of Spartineta from Hythe to Hurst Castle were made by the R.A.F. for Professor S. Mangham in 1923 - 1926, and these have been used as evidence for the course of "die-back" on the south coast, taken in association with later R.A.F. surveys. A key to the photographs available is provided as appendix 1.

Chapman (1941) separated the salt marshes on the south,

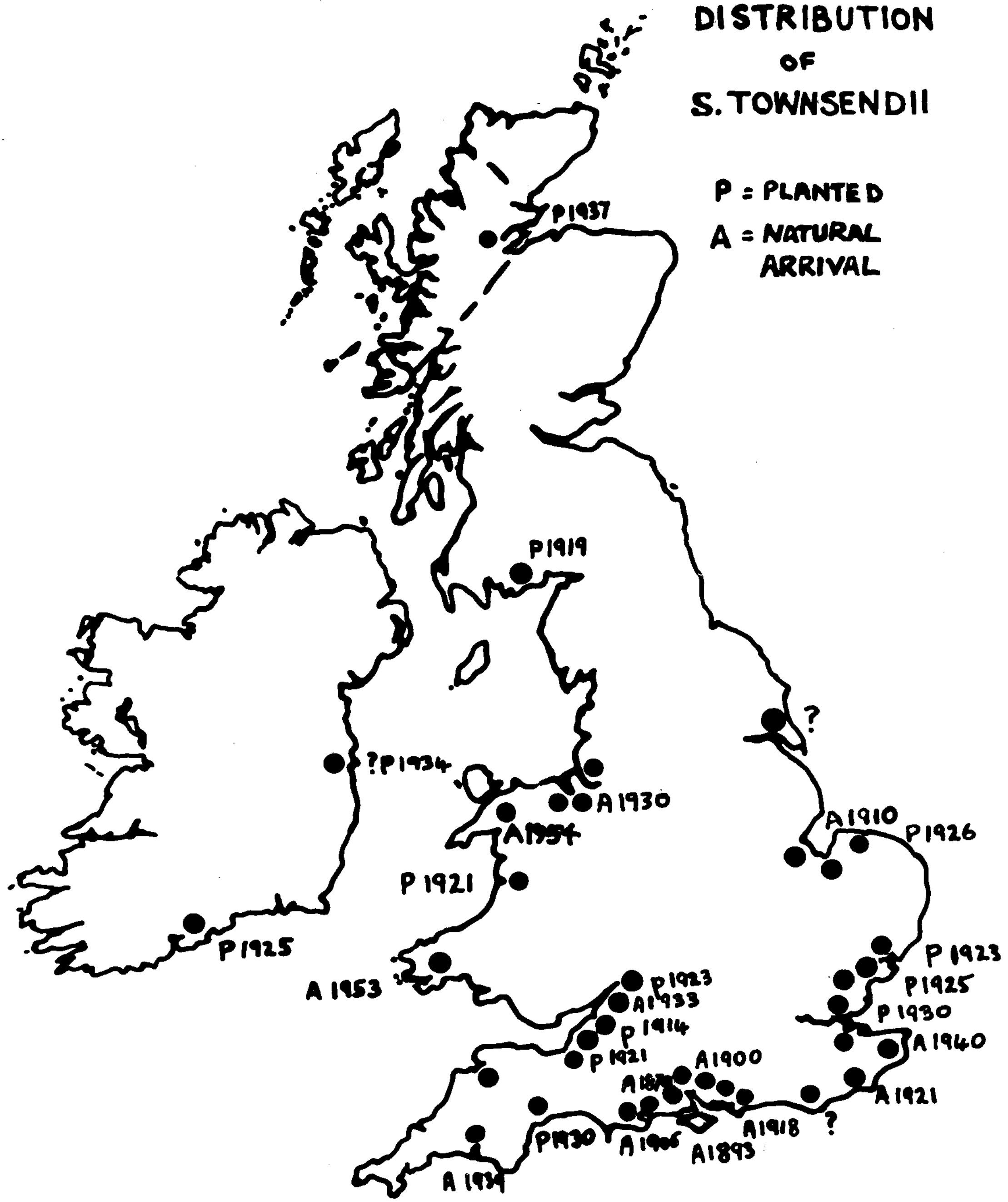


FIG. 5. Map of Spartina sites in the British Isles, with dates of arrival by natural spread (a) or planting (p) (see Appendix 2). Note south, east and west coast marshes.

east and west coasts by the characters of their soils, and the Spartineta may be distinguished in this way (Fig. 5). It is convenient to consider first the south coast marshes where Spartina townsendii originated, and the substrate is fine particled; next, the rather similar, though more variable, east coast marshes; and finally the somewhat different, sandy, west coast marshes.

1. South coast marshes.

The south coast marshes are primarily estuarine, and mainly confined to the Hampshire Basin, where they are sheltered by the Isle of Wight (Fig. 6). Being estuarine, the main source of supply of material for marsh build-up is fine silt and clay from the rivers. Locally there are shingle banks. Supplies of sand are practically absent.

The south coast marshes are typically fed by a main channel which, passing landward, divides repeatedly into lesser creeks, the ultimate ramifications of which interconnect like the capillary veins of a foliage leaf. In this way the marshes are divided into areolae or compartments, separated by creeks (Oliver, F.W., 1925).

After the first collection of Spartina townsendii in 1870 the east and west spread took place naturally to all estuaries and harbours from Plymouth to Rye, along the south coast. Only in the Beaulieu river was it planted (Appendix 2).

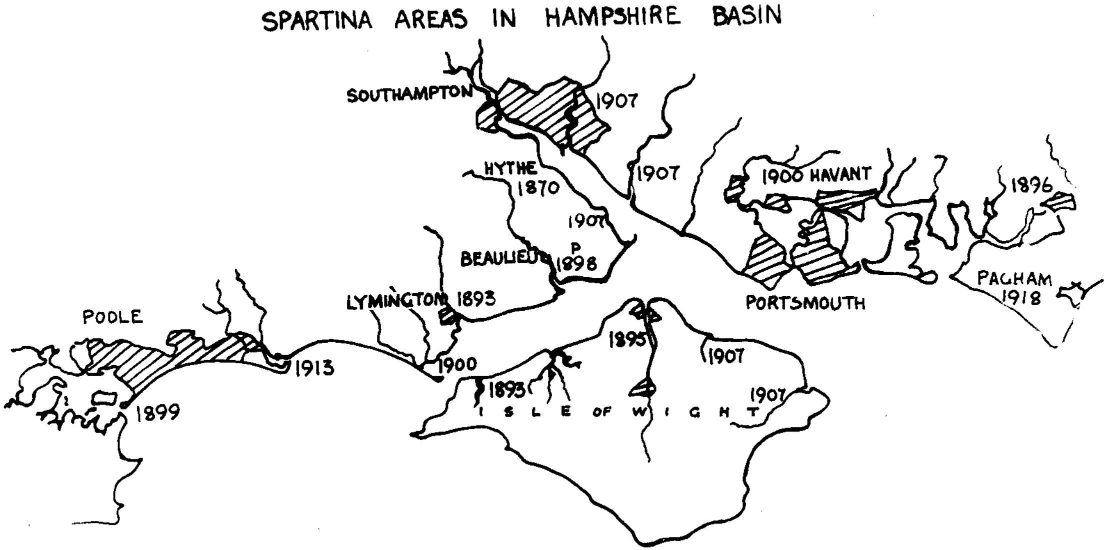


FIG. 6. Map of South Coast Spartineta, with dates of arrival by natural spread (a) or planting (p) (see Appendix 2). Note early spread especially in Hampshire Basin.

S. townsendii is the predominant plant on the south coast marshes, other plants being found only locally. In some of the older marshes *Halimione portulacoides* grows along the sides of the drainage channels. Other species associated with *Spartina* locally on the south coast include *Limonium* spp., and, on the landward sides of the marshes, *Puccinellia maritima* and *Salicornia* spp. In places where fresh water is entering or lying on the marsh, *Scirpus maritimus*, *Juncus maritimus*, and *Phragmites communis* are found. No replacement of Spartinetum appears to be occurring on the south coast.

Spartina was killed in an area at Totton in 1924 in a single extensive case of pollution on the south coast when creosote was allowed to flow over the marsh at high tide. The outflow has since been restricted to low tide, and the area has recovered. Occasional patches of oil are seen on plants along the *Spartina* front at the river mouths in the Solent, but with the possible exception of Southampton Water, pollution seldom seems of any importance in killing *Spartina*.

In contrast, patchy degeneration - "die-back" - is widespread on the south coast, extending from Poole to Chichester, in all the oldest Spartineta. It was first reported from Beaulieu in 1930 (Mills, D. in litt.). Shortly afterwards, "die-back" was noticed along the channel side at Lymington (Cooper, S. verbal communication).

Austwick (1950) also found "die-back" at Keyhaven. During the present survey, "die-back" has been seen at Havant, Poole, and Portchester, and it has been reported from West Wittering in Chichester Harbour, (Buckle, O., verbal communication).

Individual surveys of the south coast Spartineta where "die-back" has occurred were made during the general survey. These are described below.

(a) Lymington.

The estuary of the river at Lymington is approximately 2 miles long. It broadens from 100 yards across at the upper end, near Walhampton, to a width of 1½ miles at the mouth. The mud flats extend over the whole area and on either side, towards Beaulieu in the east, and towards the Hurst shingle bank, which shelters both it and the Keyhaven sward, to the west. The whole area is tidal, with a double high water, and a fast, strong scouring ebb.

The river rises north-west of Brockenhurst, widening into the tidal estuary as it reaches Lymington. It flows in a south-westerly direction through the Spartinetum, with a single loop taking it to the east for half a mile in mid-estuary.

The river has cut low "cliffs" along the edges of the Spartinetum, while in the main channel itself, dredging is carried out to allow the ferries to pass regularly up and down river.

In the upriver areas the substrate is an exceptionally soft wet silt and clay. In no other area was such a soft wet substrate encountered. Very local areas in the river are of silt and fine sand.

Spartina fills the estuary, arriving in about 1896 (Cosmo Melville, J., 1906; Linton, E., 1906), and growing with fair success until 1935. Puccinellia maritima, Salicornia spp., Phragmites communis and Juncus maritimus grow at the landward edge of the sward, but are nowhere replacing it.

"Die-back" was first noticed in 1939 (Cooper, S., verbal communication), although it actually started a few years earlier (see below), and has been on the increase since that time. The area is now seriously affected by it, 90 acres of Spartinetum having been lost, and another 500 acres in the sward having undergone "die-back" in patches. In the upper river wide expanses of bare mud are left, especially on the eastern bank, where "die-back" has been most severe. There is a great amount of "die-back" in the sward itself, in shallow "pans" which lie between the drainage channels and hold water between tides (Austwick, P.K.C., 1950). In no other area was "die-back" found in such severity as at Lymington.

(b) Beaulieu.

The estuary of the river at Beaulieu is 5 miles long, and ¼ mile wide at the mouth, where it is deflected to the eastward by a shingle spit. On either side of the river are high mud cliffs. The river is not dredged.

Planted in the river in 1898 (Mills, D. in litt. 1931), Spartina was important in protecting the shore against erosion (Montagu of Beaulieu, 1907).

"Dying out in patches, especially along the foreshore" was reported in 1930 (Mills, D. in litt.). Resurvey of the area and mapping from air mosaic photographs by the present author have shown that "die-back" at Beaulieu is negligible, though a little occurs along the channel side, and between drainage channels in the marsh itself. There is no sign that the original "die-back" area has recovered. It is therefore concluded that there has never been extensive "die-back" at Beaulieu.

(c) Keyhaven.

The Spartinetum at Keyhaven forms a triangle protected on the western side by the Hurst shingle bank. Two deep channels run through the sward parallel to the shingle. The sward close to the shingle bank is on a firm substrate, but farther from the bank, the substrate becomes softer.

Spartina arrived at Keyhaven in 1903 (Stapf, O., 1908), and now extends from the Hurst bank eastwards, where it joins the Lymington river sward. Although Salicornia spp. and Puccinellia maritima grow on the landward margin of the sward, there is no sign of any replacement of the Spartinetum.

"Die-back" was first reported from Keyhaven by Austwick, in 1950, but comparison of air mosaic photographs of 1926 and an aerial oblique photograph of 1949 has shown that "die-back" was in an advanced state before 1949. "Die-back" has occurred along the upper reaches of both channels, most noticeably on the eastern bank of the east channel. "Die-back" in the sward occurs in "pans" between the drainage channels, most often in the sward farthest from the shingle, where the substrate is softest. Though "die-back" is well advanced at Keyhaven, it is not as severe as at Lymington.

(d) Havant.

The Spartinetum at Havant extends along both shores of Langstone Harbour, the stretch of water separating Hayling Island from the mainland. The substrate of the harbour is soft mud, running out from the land with scarcely any cliff, to the undredged channel.

Spartina arrived in Havant in 1900 (Stapf, O., 1908), and now extends towards Portchester in the west, and Emsworth in the east. Salicornia spp. grow on the landward of the marsh, but there is no sign of any replacement of the Spartinetum.

"Die-back" in "pans" in the sward was first seen in 1953 by the present author, apparently being absent in 1950

(Perraton, C.J., 1953). It has also occurred along the Spartina front, towards the main channel separating Havant from Hayling Island. "Die-back", though locally severe in the Havant area, is not at present widespread.

(e) Poole.

Poole has an almost land-locked harbour, with several inlets and islands. The substrate is extremely variable, from a fine silt and clay along the western shore, to sand at Sandbanks, near the mouth on the east. The harbour is undredged, the channels remaining clear despite the accretion by the Spartina on the banks, which must reduce the scour of the harbour (Oliver, F.W., 1920).

Spartina arrived in Poole Harbour in 1899 (Stapf, O., 1908), and now extends along almost the whole of the one hundred mile shoreline.

Particularly strong growth of Spartina is found at Sandbanks where the sand is covered by a thin layer of silt and clay, which has evidently been accreted by the Spartina itself. Where the sand is not covered by mud, at the front of the sward at Sandbanks, which is still undergoing colonization, dwarfed growth of Spartina is found, apparently in response to the substrate.

"Die-back" in "pans" in the sward between the drainage channels is found on the silt and clay substrates of the western shore of the harbour. "Die-back" along the Spartina front facing the main channels is practically absent in Poole. Altogether the outbreak of "die-back" in Poole is not severe.

(f) Portchester.

The Spartina sward at Portchester lies along the north shore of Portsmouth Harbour, the area of water between Portsea Island and the mainland. On the east it is joined to Langstone Harbour by Hilsea creek. The substrate is of silt and clay, the banks extending down to the main channel with no very distinct mud cliff.

Spartina arrived in Portchester in the early 20th century, but the exact date is apparently not recorded. It now extends along the north shore of Portsmouth Harbour and up the river towards Fareham on the west, and towards Havant, along Hilsea creek, on the east. In some places plants of the general salt marsh community are found to the landward, but there is no sign of the Spartina being replaced.

"Die-back" was seen at Portchester in the sward in "pans" between drainage channels, in 1954, and along the Spartina front, towards the main channel, in 1955. At present the outbreak of "die-back" at Portchester is not severe.

(g) West Wittering.

West Wittering is a sheltered area in Chichester Harbour, on the south east. "Die-back" was reported there in 1955, having apparently occurred recently (Buckle, O., verbal communication). The area has not been surveyed.

2. East Coast marshes.

The east coast marshes are of two kinds. Those in the south are estuarine marshes, as in the Essex Stour, and resemble the south coast marshes in their sources of supply of building material, and hence in their fine particled substrate, of silt and clay. The northern east coast marshes, mainly in Norfolk, are generally developed behind shingle bars, as at Blakeney, and Scolt Head. Here, while the mainland marsh is of a firm clay or silt, behind the shingle bars the marsh is built on shingle or sand (Chapman, V.J., 1941).

Shingle barred marshes are at present either too young to show any great complexity, or the swards are too narrow. It is only in the estuarine areas that complex drainage networks have been seen. Creeks dissect the marsh, and the drainage systems appear to be developing by running together of the former Zostera pools between the Spartina patches. The biggest channels split again and again in foliage leaf capillary fashion, like those of the south coast marshes.

On all the east coast marshes, from Dingwall (Scotland) to Pegwell Bay, Spartina was planted, except at Pegwell itself, and at Scolt Head. The earliest planting was in the Wash, in 1910 (Swann, E.L., 1950), and the Spartinetum covers many hundreds of acres in the area. No other plantings were carried out until 1923, in the Essex Stour (Bingley, J., verbal communication). (See also Appendix 2). The sward in the Stour estuary is now well developed. There is no sign of succession from Spartinetum on the eastern marshes, though *Puccinellia maritima* is present, and many other salt marsh species are found associated with *Spartina* on the landward margins.

Reduced growth of *Spartina* is found in places where shingle reaches the surface of the marsh, as on the Yankee lateral at Blakeney, and at Stone Point in the Stour. Death does not occur, but only dwarfing. The phenomenon seems quite distinct from "die-back" which is sometimes accompanied by dwarfing, but which always results in death.

A single case of pollution occurred on the east coast at Brantham, in the Essex Stour, in 1950, but the area is now recovering.

Local "die-back" is found, quite distinct from the site of pollution, in the Essex Stour at Mistley, where the plants have died in small "pans". There is no "die-back" along the front, nor is the outbreak in the sward severe in this single record of "die-back" on the east coast.

3. West Coast marshes.

The west coast marshes are generally situated in wide estuaries, the Solway, Severn, Dovey and Dee. The substrates are very sandy, and mobile, the areas being capable of fast accretion, but being easily swept away by storm tides. Below the sward, silt and clay are often accreted by the *Spartina* itself, from the land washings of the rivers. The drainage patterns are little developed, though there are slightly raised banks - "levées" - beside the drainage channels in the Dovey. These apparently permanent features are, however, often washed away in storms, and new drainage patterns form. In the fast ebbing Severn the drainage channels are sharp cut, running straight down the shore.

Spartina was planted in the Severn and Dovey, and at Solway (Scotland), but apparently spread naturally to the Dee (see Appendix 2). There are considerable swards in the Severn and Dovey.

Spartina is not the primary colonizer on the west coast, a position occupied by *Puccinellia maritima*, and under heavy grazing, as in the Dovey, *Puccinellia* can invade Spartinetum. In the Dee the marsh is advancing so fast that all the plants of the salt marsh are invading simultaneously.

There was a pattern of strong growth of *Spartina* on sandy substrates which were overlain by a thin layer of estuarine silt and clay, as in the Dovey and Severn. Poor growth occurred on pure sand (cf. Poole). This compares with the observations of Spartina failure after planting on sandy substrates on the west coast of Schleswig Holstein made by Konig (1949).

On none of the west coast marshes visited was there any sign of "die-back" of Spartina.

4. Conclusions.

The survey revealed a number of general features in the status, distribution and behaviour of Spartina in Britain.

(a) Spartina is now the dominant plant of the south coast marshes, and no succession from it seems to be taking place. It occupies a strong position on the east coast, but the swards are not as well developed as on the south coast. On the west coast it occupies a position subordinate to Puccinellia maritima which is dominant on the higher marsh, and which can invade Spartinetum under grazing conditions.

(b) Spartina spread naturally to the marshes of the south coast, with the exception of that at Beaulieu, which was planted. On the east and west coasts practically all the marshes where Spartina is now found were planted with it.

(c) Spartineta are situated in sheltered places on the coast, varying from almost landlocked harbours, to estuaries, and to the lees of shingle banks. The plant apparently does not grow on exposed coasts.

(d) *Spartina* grows on the whole range of salt marsh substrates from the edges of shingle banks to sand or fine silt and clay banks.

The nature of the substrate is related to the supplies of marsh building material available, and this differs on the three coasts. On the south coast, the substrate is a fine silt and clay, with occasional shingle outcrops; on the east coast, in the south are fine estuarine silts and clays, and in the north are shingle barred marshes; on the west coast are sandy estuaries.

Very fine silts and clays which *Spartina* is capable of filtering off from waters containing them often add to the substrate after the plant has colonized it. This addition creates a characteristic top layer to the *Spartina* marsh, and is likely to be most important in the development of the substrate.

(e) Local variations in the growth of *Spartina* have been found, ranging from dwarfing, found on shingle and pure sand, to a particularly strong growth which occurs on sand overlain by the fine silt and clay accreted by the *Spartina*. These variations in growth are distinct from "die-back", no death of the plants occurring, but only variation in their vigour.

(f) Death has been caused in Spartina by occasional outbreaks of pollution, but this is not regarded as the main cause of the present "die-back". It seems that "die-back" is more widespread than the recorded cases of pollution, and areas subjected to pollution in the past have recovered. "Die-back" areas have never been seen to recover.

(g) "Die-back" has been found only in the south coast Spartineta from Poole to Chichester, and in the Essex Stour. It has not been found anywhere on the west coast. It is confined to estuarine sites and harbours, with complex drainage networks. It is of two kinds, that beside the main channels - "channel die-back" - and that between the drainage channels - "pan die-back".

"Die-back" has invariably been associated with conditions in the estuaries giving rise to very wet, soft silts and clays. These conditions are most noticeable at Lymington, where the "die-back", both beside the channel, and in the "pans", is most severe. The Lymington area was accordingly chosen for detailed survey and investigation.

III B. LYMINGTON SURVEY.

1. Description of the area.

Spartina townsendii arrived in Lymington between the years 1896 (Cosmo Melville, J., 1906), and 1903 (Linton, E., 1906). In 1904 tussocks were established in the upper river showing several years' growth (Fig. 7). By 1928 the sward was well developed (Fig. 8), and had linked with the neighbouring swards at Keyhaven and Beaulieu (air survey, 1926; Appendix 1).

Death of Spartina was caused very locally in a single case of pollution by chemical effluents in 1931, but this was quickly checked, and little damage seems to have been done to the sward (ground photographs, 1935; Appendix 1).

Austwick (1950) described the gross features of "die-back" in Lymington, and noted how it varied in character between the upper and lower reaches of the river. He described "die-back" both beside the main channel, and in "pans" in the sward. His observations have been confirmed and extended in the present work, during which a map of the area has been prepared (Fig. 9).

At the head of the river, above Walhampton, MR 33009583, depauperate plants were found in 1950 (Hubbard, C.E., in litt.). None now remain. The

FIG. 7. Lymington river Spartinetum. Ground Photograph 1904. Across King's Post mud towards Pier - note shallow mud banks with first *Spartina* tussocks.

FIG. 8. Lymington river Spartinetum. Ground Photograph 1928. Slightly oblique to Fig. 7 but towards Pier - note extensive colonization with no sign of "die-back".

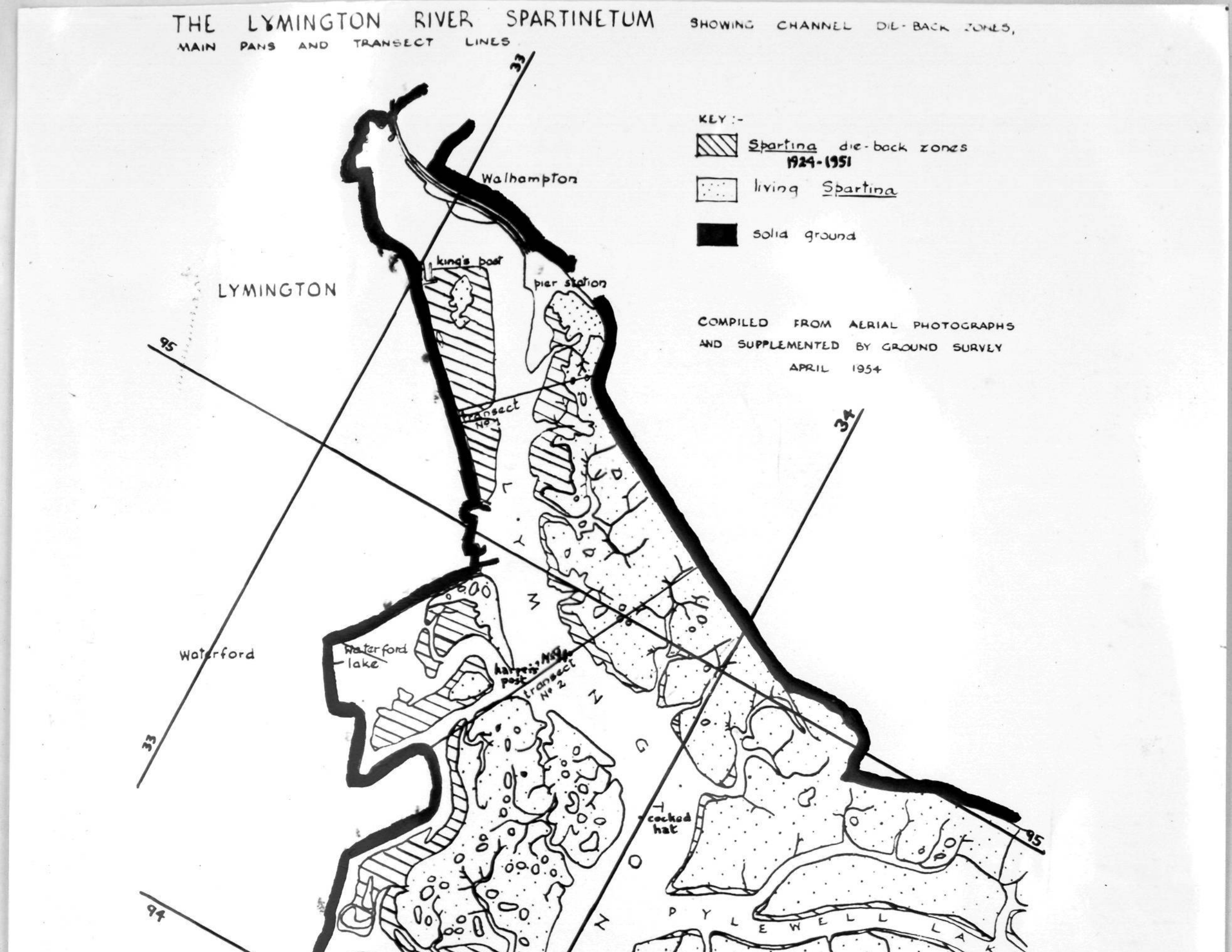


Fig. 9(a). Lymington River Spartinetum. Air Survey Map 1954.

Fig. 9(b). Lymington River Spartinetum.
Air mosaic photographs 1946.

surface is stony, and all the Spartina material has disappeared from the area. Opposite this area, on the western bank at the head of the estuary, at King's Post MR 33189535, "die-back" has left an "island" of low mud with old rhizomes in it. Much mud has been washed away from the edges of this island. A patch of slightly higher mud in the centre of it bears some living Spartina (Fig. 10). This may be compared with the previous photographs of the area (Figs. 7, 8, 10).

Behind the Railway Pier, MR 33409535, at the eastern side of the head of the estuary there is a wide zone of "channel die-back" mud with old rhizomes, having a low cliff at the channel edge. On the landward side of the bare mud are unhealthy looking Spartina plants, which are yellowing, and seldom flower (Fig. 11). This is the area where "channel die-back" is most severe. From here it extends down-river on the east bank as far as the Tar Barrel, MR 34139433, about a mile below the head of the estuary.

The sward behind the "channel die-back" zone from the Pier to the Tar Barrel is badly "panned". Near the Pier was located the most severe occurrence of "pan die-back" found in any survey. A typical "pan" is shown in Fig. 12.

"Channel die-back" is absent on the west bank between King's Post and the river mouth, though the sward behind is badly "panned". The most noticeable feature of the western bank is an area of particularly strong growth of Spartina

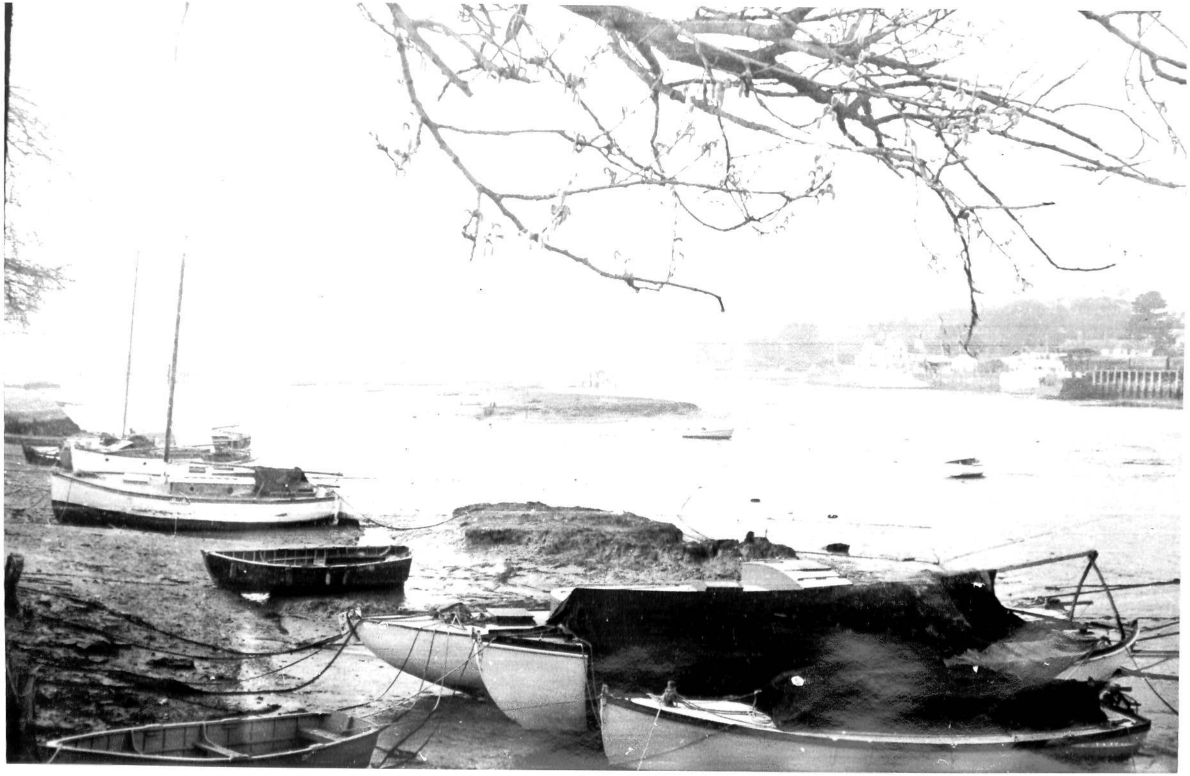


FIG. 10. Lymington river Spartinetum. Ground photograph 1956. Similar angle to Figs. 7 & 8 (q.v.). Note small mud bank with dead *Spartina* (foreground) and (left middle distance) island of "die-back" mud with small clump of live *Spartina* at far end.

FIG. 11. Lymington river channel "die-back" 1956. View across upper reach. Note unhealthy plants (foreground) giving way to "die-back" mud still containing dead rosettes, extending out to river channel (middle distance). "Die-back" mud also on opposite bank.

FIG. 12. Lymington river pan "die-back" 1956. A pan in the eastern sward (Pier area). Note thin growth on pan edges, associated with a low level. Standing water in the pan itself, with no living plants.

beside the main channel at Cocked Hat, MR 33999464.

At the river mouth, on the east bank, in the area MR 345940, the character of "die-back" changes. Dwarfed plants with torn, discoloured leaves grow almost to the channel edge. The full force of the south westerly gales is felt in this area. Gullies are forced in the sward, and turves are often thrown by the waves from the sward edge on to the grass behind. The mouth of the river has widened considerably in recent years. Much of this is due to the movement of the shell and shingle bank on the west of the river mouth. The large lake to the east of the river mouth, MR 34679445, has been continuously inundated for many years, without apparently undergoing any change.

Austwick (1950), noting the features of "die-back", sketched them, freehand, on a map. Accurate re-survey has been carried out using recently available photographs (Appendix 1). Not only has it been possible to confirm the findings of the ground survey, but it has been possible to map both "channel" and "pan die-back", and, in most areas, to date their appearance with some accuracy.

The available photographs have shown that there was little "channel die-back" between 1928 and 1934, during which time the pollution occurred. Most of the "channel die-back" occurred between 1935 and 1946. "Pan die-back" has been developing since 1924, and is on the increase, though some areas have apparently not changed over long periods of time.

The latest vertical air photographs show dark patches in the sward in the area of the Pier, where "pan die-back" is most severe. Previously these have been noticed in sites that have given rise to "pan die-back" within a few years. It is thus strongly suspected that "pan die-back" is still active in the region of the Pier. By contrast, "channel die-back" appears to be increasing hardly at all at the present time.

On the basis of this survey of the Lymington area, four sites are of particular interest in connection with the "site effect" noted from transplanting experiments. These are the most severely affected areas of "channel" and "pan die-back" near the Pier, the area near the river mouth where plants are torn and dwarfed, and the area of strong growth at Cocked Hat on the western bank. These sites were chosen for further investigation.

The areas of "die-back" near the Pier were those that had been suggested as contributing to the dredging requirement. It is of interest to note that the dredging requirement fell from 80,000 cu. yds. in 1946 - 1949 to 42,000 cu. yds. in 1949 - 1956. It seems likely that the original comparison of requirements (Table I) was not a fair one over a longer period of time. The physical system has recently been altered, however, by the introduction of a new ferry which more efficiently scours the channel. It was decided to investigate any connection between "die-back" and the physical features of the habitat by accurate surveys in the upper river.

2. Transects.

As an integral part of the survey of the Lymington Spartinetum, and to gain a fuller understanding of the processes at work, two levelled line transects were made across the river channel where "channel" and "pan die-back" were most severe, one in the upper reach, and one towards the middle of the estuary (Fig. 13).

Relative levels were accurate to within ± 2 cms., but the unstable substrate may have caused errors due to "tilt". The channel profiles were completed by using echo sounding records made by Mr. Franks, Hydrographic Survey Officer, Docks and Inland Waterways; and by the Royal Navy. Pre-dredged and dredged profiles were available.

The upper transect was 285 metres long, and ran from behind the Pier on the eastern bank at the head of the estuary, over the "die-back" zone of the eastern bank, across the river channel, and over the "die-back" zone of the western bank. Spartina was present on the eastern side of the transect, but had completely died back from the western side.

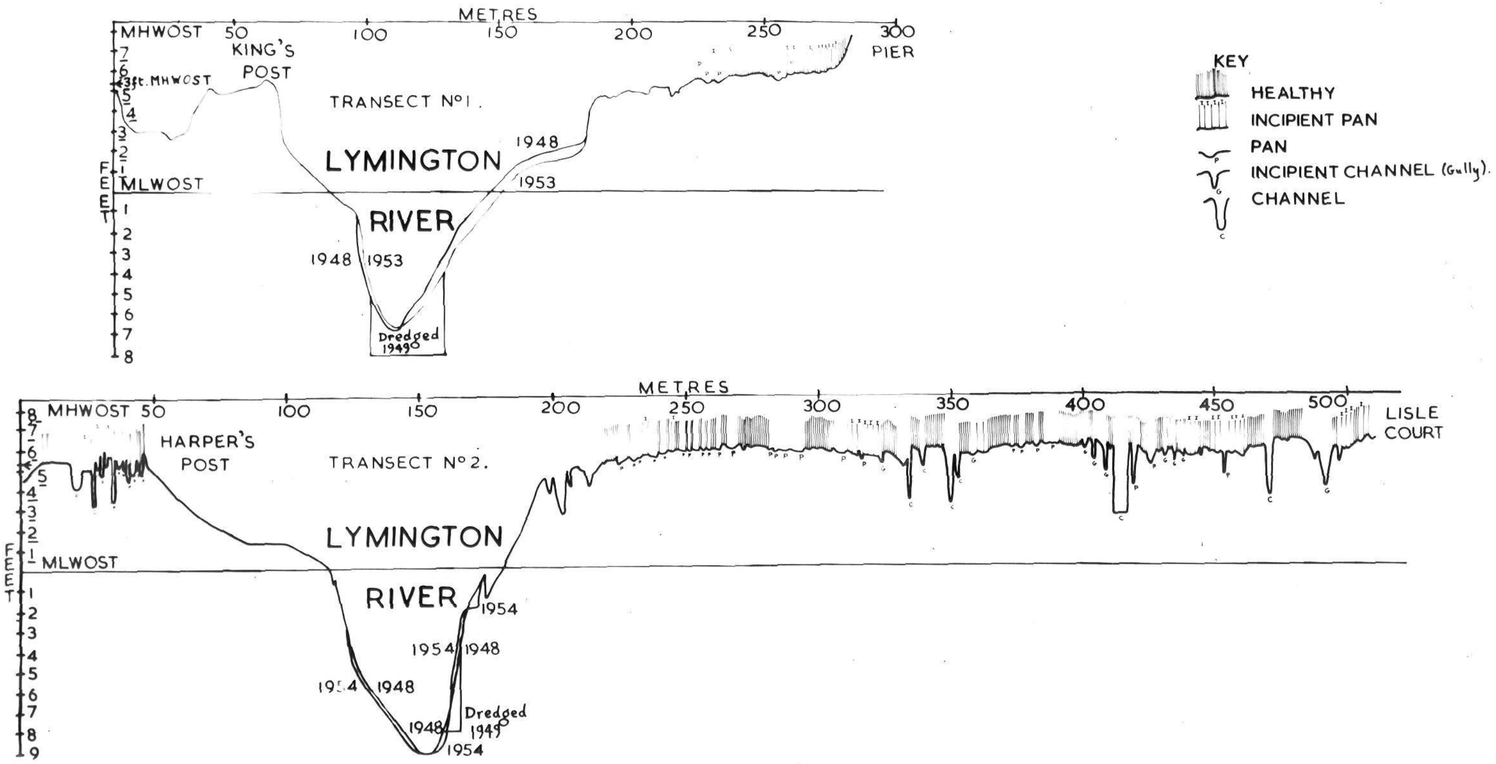


FIG. 13. Two levelled line transects in the Upper Reach at Lymington. Across the channel "die-back" zones, and the unhealthy sward. Note the low level of channel "die-back", pan "die-back" (P), the relatively low level of incipient pans (I) and the higher level of healthy growth of Spartina: also the dredging changes in the river for the period.

On the eastern side of the valley, *Puccinellia maritima* and *Salicornia* spp. grew on the highest mud, near the level of Mean High Water Ordinary Spring Tides (MHWOST), with *Spartina* on lower ground, where it would be immersed for a longer period on each tide. The height of the pans found in the eastern sward above MLWOST was greater for those at the back of the sward than for those nearer the channel. The level at which pans occurred was sloped down the shore, like the sward itself.

The sward ended, on the channel side, in dwarfed, yellowing plants, merging into the old stumps of the "die-back" mud in the eastern "channel die-back" zone. This "die-back" zone was just below the level of 3 ft. immersion at MHWOST, and ended in a cliff to the deep water channel.

On the western shore, at King's Post, the bank rose steeply from the deep water channel, to just above the level of 3 ft. immersion at MHWOST. No living *Spartina* occurred on the west bank at this point. That the area had been colonized formerly was evident from the standing *Spartina* stumps.

The lower transect, of 600 metres, ran over a drainage system in the Spartinetum, across the main channel, and to one side of Waterford Lake, towards the tip of the reclaimed land beside the lake. *Puccinellia maritima* and *Salicornia* spp. grew on the landward margin of the sward to the east. In the sward the system of drainage channels complicated the pattern of the pans. In this transect, the level of the pans rose between each channel, instead of only rising away from the main channel, as was seen in the shorter transect where there was no accessory drainage system. The lake where "die-back" occurred was found to be below the level of 3 ft. immersion at MHWOST.

Despite the greater complexity of the second transect, the two had many features in common and between them showed the following significant points:-

(a) The Spartinetum formed a pure community except at the highest levels, towards the landward edges, where Puccinellia maritima and Salicornia spp. occurred. This mixed community was subjected to trampling by ponies and the Spartina in it appeared to be preferentially grazed.

(b) There was a uniform pattern of more vigorous Spartina growth at the higher levels, usually those above the level of 3 ft. immersion at MHWOST. (The Spring Tide range being approximately 8 ft.).

(c) In the main sward, the "die-back" appeared to be consistently associated with pan formation. The main pans, which hold water continuously, have old decaying Spartina rhizomes in their mud floors. Areas of "incipient panning", with the Spartina at a relatively low level and tillering with reduced vigour, often occurred between the main pans and the drainage channels. In many cases gullies connect the pans to the nearest drainage channels.

(d) The "die-back" pans rose in level as they became further removed from the channels; this was associated with a corresponding rise in the minimum level of vigorous Spartina growth, which was always slightly above the level of adjacent pans.

3. Discussion.

Whereas the general survey showed that "die-back" was essentially a feature of the south coast Spartineta where soft, wet substrates were found, and particularly that at Lymington; the Lymington survey revealed that there were four areas of particular interest in the estuary. These were the area of strong growth on the west bank, the area of dwarfed growth on the east of the mouth, the area of "channel die-back" near the Pier, and the area of "pan die-back" near the Pier. Both of the areas of "die-back" were definitely associated with low levels in the Spartinetum.

"Channel die-back", on the edge of the Spartinetum, occurs at a level very near 3 ft. (or 6 hrs.) immersion on the high tide, and this has been suggested as a critical depth for Spartina growth (Oliver, F.W., 1925). Jacquet (1949), however, regards this as variable with salinity and other factors, and this may explain the slight variations in the depth of immersion at which "channel die-back" occurs.

Subsidence could bring about immersion to the critical depth, and Austwick (1950), and Mangham (in litt. 1950) have suggested that dredging might increase subsidence. "Channel die-back" occurs in both dredged and undredged estuaries, so that dredging may not be important. The eastern banks of the estuaries are often more severely affected by "channel die-back" than the western banks, and this suggests that some other physiographic factor such as sedimentation, indirectly affected by the prevailing south westerly wind, may be at work. The significance of these observations on "channel die-back" is not fully understood.

"Pan die-back" is found in the compartments of the marsh separated by the creeks of the drainage system. Beside the creeks raised banks impede drainage, and this is associated with poor tillering and "incipient panning". Further hindrance of drainage may convert the incipient pans into "die-back" pans, in which the Spartina has died out completely. There was no evidence that Spartina "die-back" led to the formation of the pans, but all the evidence was in favour of the pans giving rise to "die-back", apparently as a result of the poor drainage.

Gullies are often found leading from the pans to the nearest drainage channels. These have been seen to be formed by the breakdown of the pan wall by the tidal force. Some gullies are deep enough to effect complete pan drainage, when some recolonization may occur. Sometimes the gullies delimit "butts" which have slipped into the drainage channels, and this contributes to pan breakdown. The Spartina on the butts often decays. By pan formation in the areas between drainage channels, and by later breakdown, degradation of the sward may proceed faster than colonization, and progressive "die-back" sets in.

A detailed investigation of the physiographical and ecological processes at work in the Spartinetum is being made by Miss E. M. Uren. The present work was continued as a study of the effects of the processes outlined. In the "die-back" sites growth failures had already been noted, and attention was directed to these. Growth of Spartina in the four sites of interest at Lymington was made the subject of a detailed investigation.

IV. EXPERIMENTAL MORPHOLOGY.

A. Introduction.

A full study of the biology of Spartina townsendii was suggested by Austwick (1950) to explain the processes of building and maintenance of the sward. It was further regarded as necessary to explain the manner in which "die-back" was occurring, and to reveal any possible physiological basis of it. The performance of some few perennial herbage grasses can now be predicted as a result of recent advances in grassland technology (Langer, R.H.M., 1956), but the behaviour of wild grasses is almost completely unknown, and for the investigation of S. townsendii a fundamental approach had to be adopted.

B. Methods and Materials.

In studying the development, growth and perennation of S. townsendii,[2] three techniques were used:-

1) The development of seedlings was observed in culture.
2) Field observations were made, and collections examined.
3) Small scale field trials were carried out.

---

[2] See Introductory footnote - fertile material was used for all morphological studies.

Seed material was collected from Whippingham, Isle of Wight, an area free from "die-back", in November 1953, and stored in humidity chambers, to ensure prolonged germinability (Nelson, A. & Munro, J., 1934). Storage was carried out at various temperatures.

Field collections of plants for morphological examination, and field trials, were carried out principally at Lymington.

C. Results.

1) Development of seedlings.

(a) Germination.

The embryo is viviparous, and germination takes place in a few days from stored, or naturally overwintered, seed. Germination of fresh seed takes longer and is less uniform. In germination the coleoptile emerges from between the persistent flowering glumes, and this is followed shortly afterwards by the appearance of the coleorhiza from the other end of the mesocotyl. The first leaf penetrates the coleoptile; the seminal root pushes through the coleorhiza; and the seedling is established (Fig. 14).

Preliminary experiments showed that very few seeds germinated in pots or on filter paper watered with sea water, but that up to 40% germination occurred in fresh

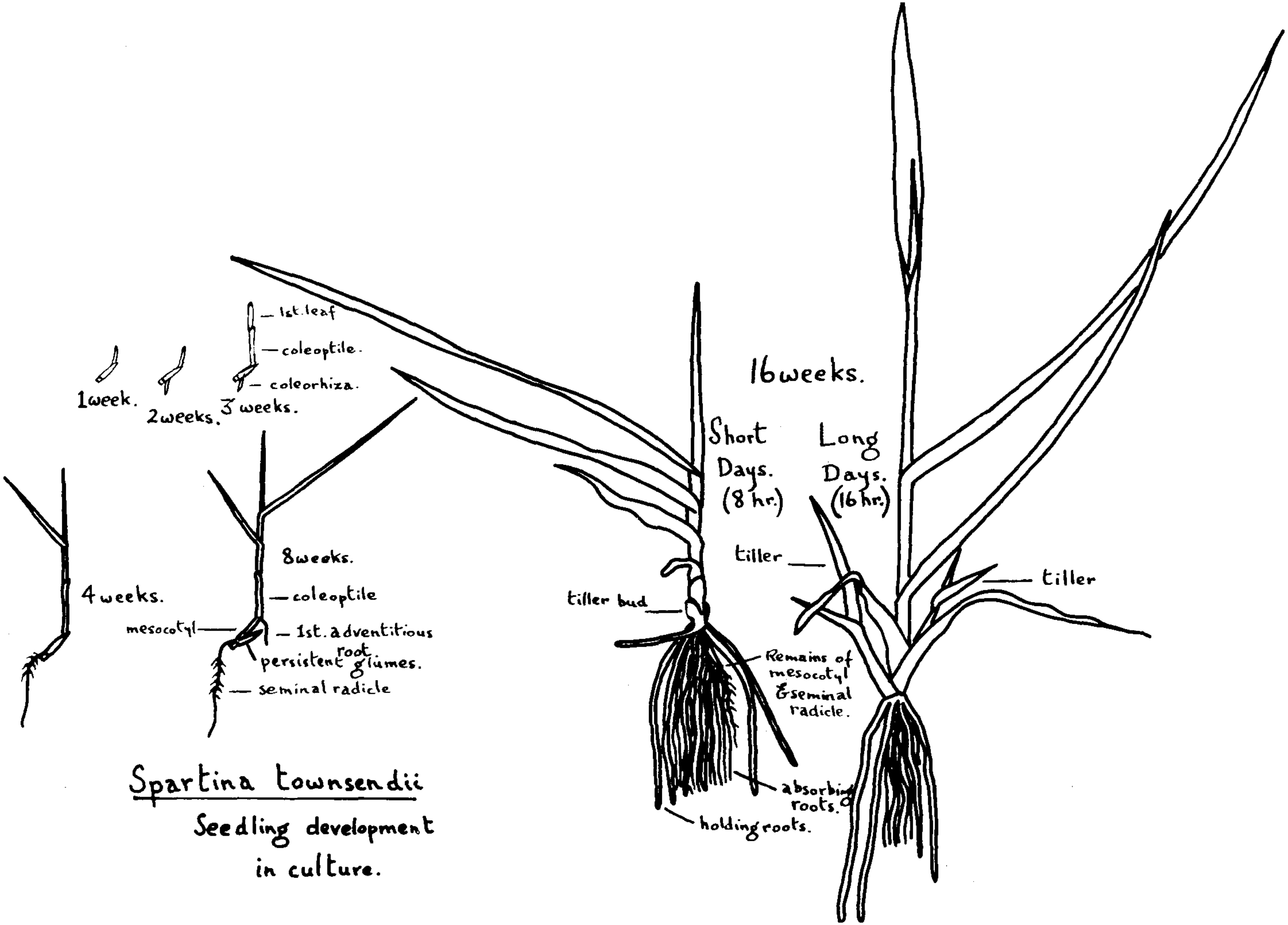


FIG. 14. Spartina seedling development in culture. Note coleoptile appearance first ("false germination"), followed by coleorhiza development; first leaf penetration; seminal root, and finally adventitious root development.

water, an observation of a type which has been made on several halophytes (Jacquet, J., 1949). Only 5% of the seedlings lived beyond the 15th week, but germination was raised to 75% and survival to 60% after storing over winter at 5°C. the fresh seed collected in November.

(b) Seedling growth.

Preliminary experiments in growing-on seedlings showed that under laboratory conditions uniformly successful growth could not be obtained by watering with sea water, the leaves becoming encrusted with salt, and the plants dying. Jørgensen (in litt., 1955) has succeeded in growing plants in sea water with a daily immersing mechanism to simulate tidal movement, but this was impracticable on the large scale of seedling culture envisaged in this experiment.

Some seedling failure also followed growth in potting soil, the heavy watering necessary for Spartina growth resulting in soil deterioration, which apparently caused the death of some plants.

Successful growth was obtained by transferring the seedlings to pots of sand, and watering daily with rain water, and once weekly with Knop's solution, with a trace of manganese added (as used by Purvis, 1934). To retain the moisture the pots were stood in saucers. Seedlings were grown on, both indoors in the temperate greenhouse, and outside.

The seedlings grew by producing new leaves which emerged through the sheath of the next older leaf. The oldest leaves had distinct sheaths and, standing at an angle from them, flat, ribbed, laminae. The younger leaves were no more than hoods over the vegetative apex. Seedlings outdoors produced an average of 6 leaves in 25 weeks starting in June 1954, while those indoors produced an average of 9 leaves in the same time. The plants grown indoors were lighter in colour and somewhat limper than those grown outside, but were otherwise quite healthy. The development of indoor plants was followed in the later stages as it was faster than that of outdoor plants.

In the axil of each leaf and of the coleoptile are three microscopic buds, two of which may form roots, the third of which may form a new stem. At the three leaf stage, after 8 - 10 weeks, the root buds at the base of the coleoptile usually begin to grow out adventitiously. These take over the functions of the seminal radicle, which, together with the mesocotyl, normally withers at the 6th leaf stage, after around 20 weeks (Fig. 14).

(c) Leaf production.

Germinating seedlings, some of which had been stored at 5°C. and others which had been stored at 15°C. for 6 months, were grown on; some in 16 hr. days, and others in 8 hr. days, in the greenhouse. There were initially 60 seedlings undergoing each treatment. Sampling and

TABLE V.

Average number of leaves on main stems.

	Treatment	5wks	10wks	15wks	20wks	25wks	30wks	35wks	40wks	45wks	50wks
5°C;	16 hr. day	1.9	3.0	5.0	8.7	10.0	11.0	13.0	15.0	16.0	16.0
15°C;	16 hr. day	1.2	2.0	4.0	7.0	9.0	9.0	11.0	13.0	13.0	15.0
5°C;	8 hr. day	1.9	3.2	5.0	7.3	9.3	11.0	12.0	13.0	13.0	15.0
15°C;	8 hr. day	1.5	3.0	4.5	7.5	9.0	all died		-	-	-

occasional deaths reduced this until there were only two remaining in each of three treatments, and none in the fourth, at 50 weeks.

The number of leaves on the main stems of the plants was counted at 5 weekly intervals (Table V).

The rate at which leaves were produced was substantially unaltered by the cold and daylength treatments. Under these conditions leaf number at any time was related only to age of the plant (Fig. 14).

Leaf production ceased when flowering occurred, after a year's growth, in both the 5°C and 15°C long day treatments. No flowering occurred in short day treated plants, leaf production continuing until the end of the experiment, 10 weeks later.

(d) Main stem growth.

Main stem growth was greatly affected by the daylength treatment. Main stems averaged 33.3 cm. in length after 50 weeks of 16 hr. days, but only 7.5 cm. in length after 50 weeks of 8 hr. days, after 5°C storage.

Transferring the plants from long days to short days caused them to produce short internodes, while transferring plants from short days to long days caused them to produce long internodes.

Main stem heights under these conditions reflected not the age, but the daylength treatment the plants had received (Fig. 14). It is not known whether the differences observed were due to a true photoperiodic effect or to differences in the total light quantity received by the plants. This point of uncertainty did not invalidate the interpretation of later results.

(e) Vegetative reproduction.

The capacity for vegetative reproduction lies in the centremost of the three miscroscopic buds in the axil of each leaf of the main stem. The bud groups are arranged alternately up the stem, the divergence angle in Spartina being nearly 180°. At the three-leaf stage of the main stem not only do the two outer, root buds in the axils of the lower leaves begin to develop, but so do the central, stem buds. The stem buds are shield-like in shape, and thin in section, covered by a double keeled first leaf. The first bud usually becomes visible to the naked eye after 15 weeks summer growth, when 5 leaves have formed.

The first visible bud may not be from the axil of the coleoptile, but from that of a leaf above. The bud is usually an upwardly directed, tiller bud; not at first penetrating the sheath subtending it, but growing a short distance inside it (intravaginally), forming a small angle with the parent stem (Fig. 15). It finally emerges to repeat the developmental history of the main stem; producing

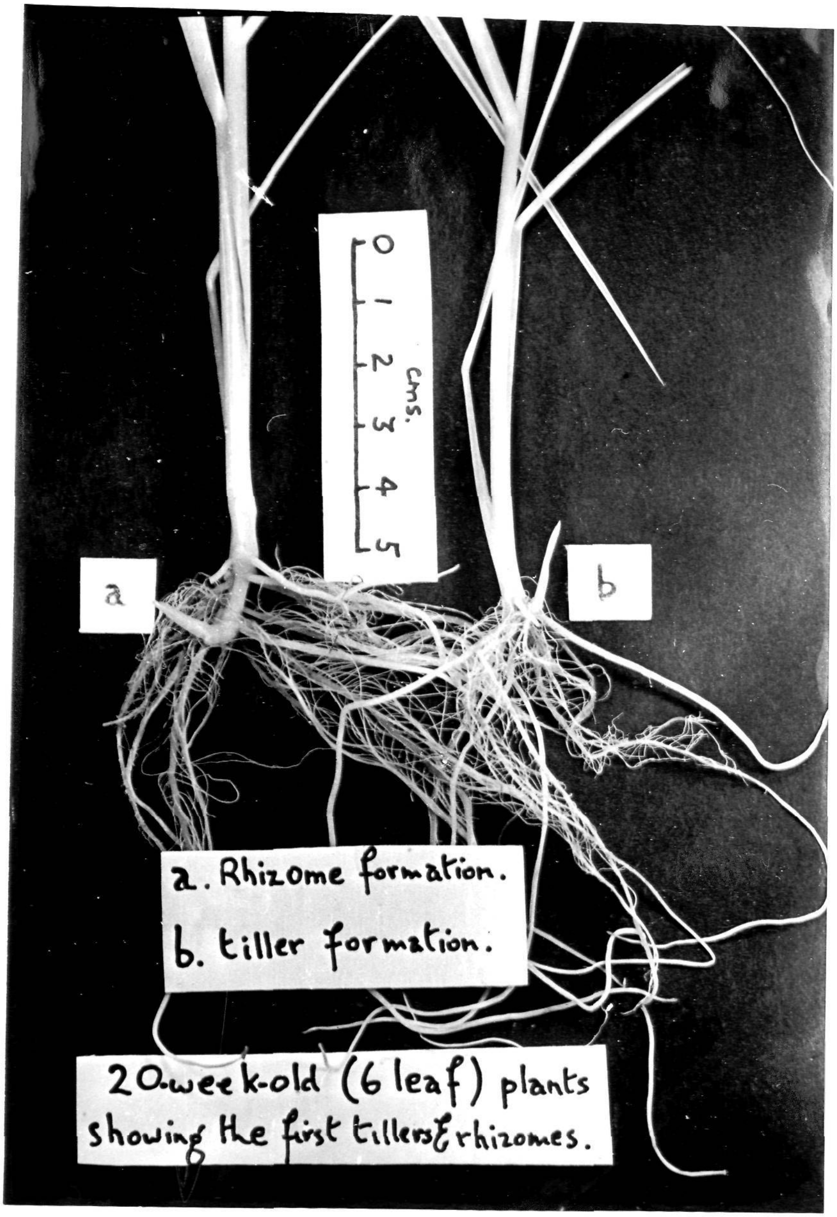


FIG. 15. *Spartina* tiller and rhizome development in culture. Note intravaginal, upright tiller growth and extravaginal, downward rhizome growth. Tillers finally leafy; rhizomes white and awl-like.

on its higher nodes leaves with flat green laminae; and eventually terminating in an inflorescence.

Sometimes the first visible bud is from one of the very lowest nodes, and it is downwardly directed, immediately penetrating the leaf sheath (extravaginal), forming an angle greater than the perpendicular with the parent stem and remaining colourless and awl-like, with white scaly leaf rudiments (fig. 15). Usually buds do not grow out in this way, as rhizomes, until at least 10 leaves have formed on the main stems (under culture conditions), and the plants are 25 weeks old, with some tillers already growing.

It is always found that the lowest buds on the main stem, and when this is not perpendicular, those on the lower side, form rhizomes; while those higher up, or on the upper side, form tillers, or sometimes short, quickly upturning rhizomes. It may be that this is due to a gravitational effect, as has been found in <u>Eriophorum</u> (Phillips, M.E., 1953).

The numbers of tillers and rhizomes formed during the day length experiments were counted, and averages calculated (Table VI).

In short days large numbers of tillers formed, while in long days there were few. Like the main stems, tillers grew long in long days, but remained short in short days, while their rate of leaf production was the samein both

TABLE VI.

Average numbers of tillers and rhizomes.

Treatment		10wks	15wks	20wks	25wks	30wks	35wks	40wks	45wks.
5°C; 16 hr. day	t	0.0	0.5	4.0	5.0	5.0	7.5	10.0	10.0
	r	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3
15°C; 16 hr. day	t	0.0	0.0	1.0	1.0	1.5	3.5	4.5	5.0
	r	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5°C; 8 hr. day	t	0.0	0.0	0.4	1.7	10.0	16.5	25.0	31.0
	r	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5
15°C; 8 hr.day	t	0.0	0.0	1.5	2.5	all dead -		-	-
	r	0.0	0.0	0.0	0.0	all dead -		-	-

treatments, depending only on the age of the tiller examined (Fig. 16). As in the observations on main stem growth, it is not known whether the daylength effect was one of true photoperiod or of total light quantity received.

In no treatment were there more than a few rhizomes formed, though, on average, more rhizomes formed in short days than in long days, after storage at 5°C. Being at the end of the experiment, these rhizome numbers came from very few plants, and therefore are probably not based on sufficient observations to be very reliable. Rhizoming appears to be at most, spasmodic in culture.

Tillers may propagate the plant vegetatively by developing from the leaf axils of the main stem, but this capacity is limited; firstly by the small number of potential tiller buds; the highest buds of the stems, more than 5 cm. above ground level, do not develop, and the lowest usually form rhizomes: and secondly by the nearness of the tillers to the main stem, resulting from their upright growth.

Rhizomes, however, may run underground for a considerable length of time (usually not more than one season). Any of the numerous scale leaf axillary buds may produce a new stem, though an undamaged rhizome seldom branches. If damaged, the apex is replaced by a lateral bud, and rhizome growth is almost certain to succeed in maintaining an apex until it turns upwards.

The first formed ("first generation") rhizomes from

FIG. 16. *Spartina* development in culture. Daylength effects. Note few, long tillers in long days; many, short tillers in short days. Rhizoming only spasmodic. No noticeable effect of cold treatment.

the parent stem soon turn upwards, producing green leaves on a rosette of short internodes. This rosette behaves as a new main stem, forming a shoot cluster at a distance from the parent stem, with the tillers growing from the upper and inward side, and "second generation" rhizomes from the lower and outward side.

Forward and linear growth is checked in the second generation of rhizomes, as their buds are horizontally placed and laterally directed, instead of being placed perpendicularly to the ground, as in the first generation. Gravitational forces are thus placed equally on buds of both sides. Rhizomes may form on either or both sides, and chance displacement of the buds vertically seems to decide to some extent whether tillers may form.

The third generation rhizomes grow away from the main stems peripherally, on what is now the radially expanding "tussock".

2) Field observations and collections.

(a) Formation of sward.

Onward growth of Spartina tussocks to form sward could not be observed in laboratory culture owing to the inherent difficulties in reproducing field conditions, and through limitations of space. Field observations on the later stages of tussock growth have been made by Mrs. P-A. Caldwell (unpublished).

Mrs. Caldwell found that tussocks develop an area of low shoot density in the centre, then successive rings of low shoot density toward the periphery. These "fairy rings" possibly result from gregarious flowering of shoots of similar age which, dying off, are lost. The stem density is restored by ingrowth of rhizomes from neighbouring rings - producing new uniformly aged annuli - which again flower gregariously. As many as seven rings of low shoot density may be found in a single tussock, though usually the marsh is so thickly tussocked that fusion of one tussock with the next has occurred by this time. After tussock fusion the uniformity of vegetative reproduction is lost, and an irregular non-directional system of sward maintenance rhizoming is set up.

(b) Morphology of the sward plants.

Collections were made in February 1955, and then at monthly intervals from August 1955 to August 1956, typical plants[2] being taken from the following sites chosen in the ecological survey of the Lymington estuary:-

i) The area of healthy sward at Cocked Hat in mid-estuary, MR 33999464.

ii) The area of "channel die-back" in the sward near the Pier, MR 33409535.

---

[2] See Introductory footnote - fertile material was used for all morphological studies.

iii) The area of "pan dieback" in the sward near the Pier, MR 33409540.

iv) The area on the east of the river mouth where the plants were dwarfed, with torn and discoloured leaves, at Eastlake (No. 5 Post), MR 34509405.

Detailed morphological investigations were made of the field collections.

(1) Healthy plants.

Healthy plants examined during the winter months appeared dark green, and vigorous. Main stems were only 2 - 4 cm. tall in May, being rosettes of 7 - 9 short internodes, each bearing a leaf. In the leaf axils of the rosettes were young tiller buds, and lower down, young rhizome buds, most with around three internodes. Almost every available axil contained a bud. The buds had been laid down during the winter, and the great majority (85%) had survived until May. There were also a few long rhizomes, about to turn up to form rosettes at a distance from the parent stems. In May a few tall dead stems persisted, the remains of those that had flowered in the previous year (Fig. 17).

Rapid elongation of the stems took place in early summer, the rosettes becoming tall flowering culms, beginning in July in most years. In August, these were 40 - 80 cm. tall, with 8 - 10 leaves between the basal rosette and the inflorescence. The next shorter stems, representing the

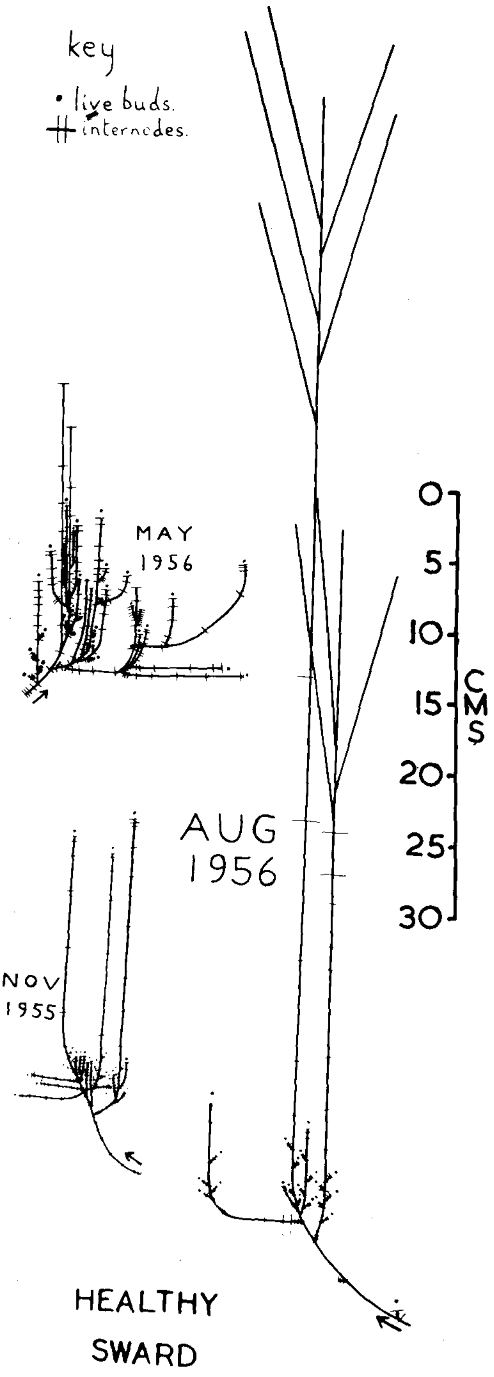


FIG. 17. Lymington healthy sward plants in May, August and November. Note high bud survival in May, with many rosettes; rosette growth providing tall August stems but few rhizomes; tallest growth in November - buds laid down and over-wintering commencing.

May tillers, were, in August, 5 - 10 cm. tall, and formed the second flowering generation of the year, usually after growing 6 - 8, or exceptionally 5, leaves above the rosette. On these short stems there was yet another generation of tillers which either flowered later in the same season, or over-wintered and flowered in the following season. There were usually few rhizomes in the healthy sward in August, most having become rosettes, and producing tillers (Fig. 17).

As individual flowering finished, the culms dried off, and by the time the first fruits were ripe, in November, the flowering stems that bore them were quite dead, being no more than straws attached to the plants, showing where they had grown. Late flowering stems, of the second or third generations of tillers, had up to 10 leaves in November, and were from 20 - 80 cm. tall before flowering. Shorter stems, of 5 - 10 cm., and with 4 - 5 leaves, represented the third tiller generation, and were probably destined to over-winter before flowering (Fig. 17).

The all-important time of the year was reached in November, when in the shortening days fewer stems were rapidly elongating, flowering, and dying. At this time of the year buds began to grow from the axils of the shorter stems. At the bases of the stems, rhizome buds formed, and at their higher nodes, tiller buds. The rhizome buds grew out to produce the rosettes of the following spring; which, together with the tiller buds, produce the bulk of the flowering stems of the following summer.

Flowering finally finished in February, so that, in that month, full stock could be taken morphologically of the shoots that had reached maturity or flowered in the previous year. Dead straws represented the matured stems. Tall stems, of 25 - 85 cm. and with up to 10 leaves might either just reach flowering stature, or would be quite likely to over-winter before flowering (Figs. 17, 18).

In February the prospects of flowering for the year were represented by the buds, laid down in November, which had survived the winter and begun to elongate. These were supplemented by the rhizomes, which, laid down in November, had remained underground unbranched in these conditions of healthy sward, and, upturning, brought to the surface apices which developed rosettes of leaves, in whose axils tillers developed to produce new shoot clusters. The tillers and clusters grew on well in the healthy conditions, to produce abundant inflorescences, on strong plants.

All collections made in the healthy area showed a most marked upright habit of growth, suggesting that accretion was occurring rapidly in this area.

Similar plants to those found in this, the healthiest area of the Lymington sward, were found in many other uniformly healthy areas, notably at Sandbanks, in Poole

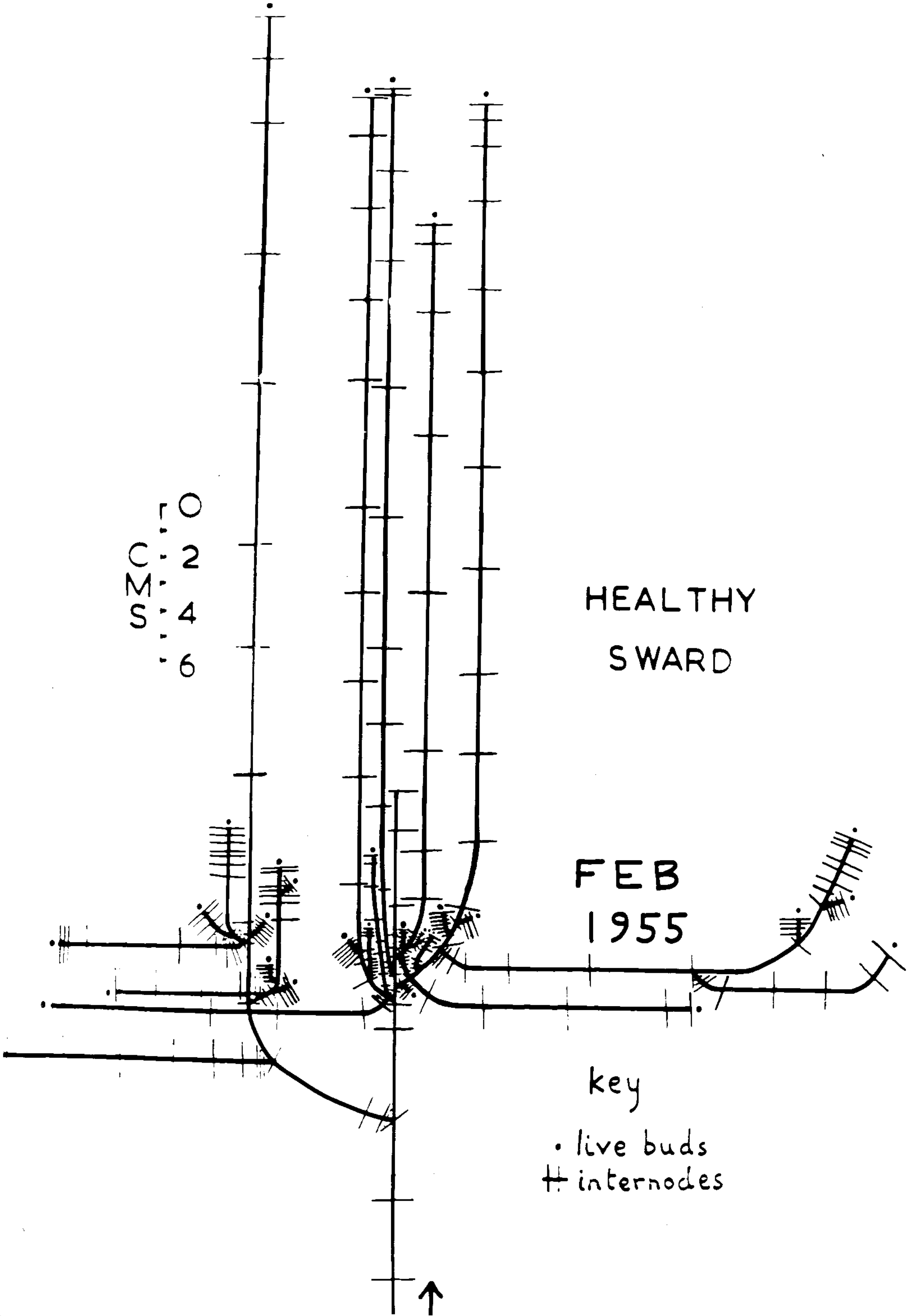


FIG. 18. Lymington healthy sward plants in February. Note strong growth and rapid turnover of material, also upright habit.

Harbour; In the Dovey estuary; and in the strongly growing areas on the east coast. In all these areas Spartina is maintaining itself by vigorous vegetative reproduction on what appear to be actively accreting substrates.

(ii) "Die-back" plants.

Plants taken from pan "die-back" sites showed essentially similar points of morphology to plants taken from channel "die-back" sites. All "die-back" plants showed the same differences from healthy plants.

"Die-back" plants examined during the winter months were often yellowish and unhealthy-looking, or actually weak-stemmed. The main stems were of rosette form, like those in the healthy sward at this time, 2 - 4 cm. tall, but with fewer leaves (3 - 6). There were in the "die-back" areas, however, many fewer rosettes than in the healthy areas, up to 90% having died. Moreover fewer buds were developed in the leaf axils of the "die-back" plants than in healthy plants. 80 - 95% of the buds that formed during the winter had died in the "die-back" plants before May. In that month in the "die-back" plants there were longer rhizomes than in thehealthy sward, up to 15 cm. long, with as many as 14 nodes. Their apices were feeble, and showing no signs of turning up to form new rosettes. In many cases the apices had died, and a "damage reaction" had occurred, lateral buds growing out and taking over the function of the dead apices (Figs. 19, 20).

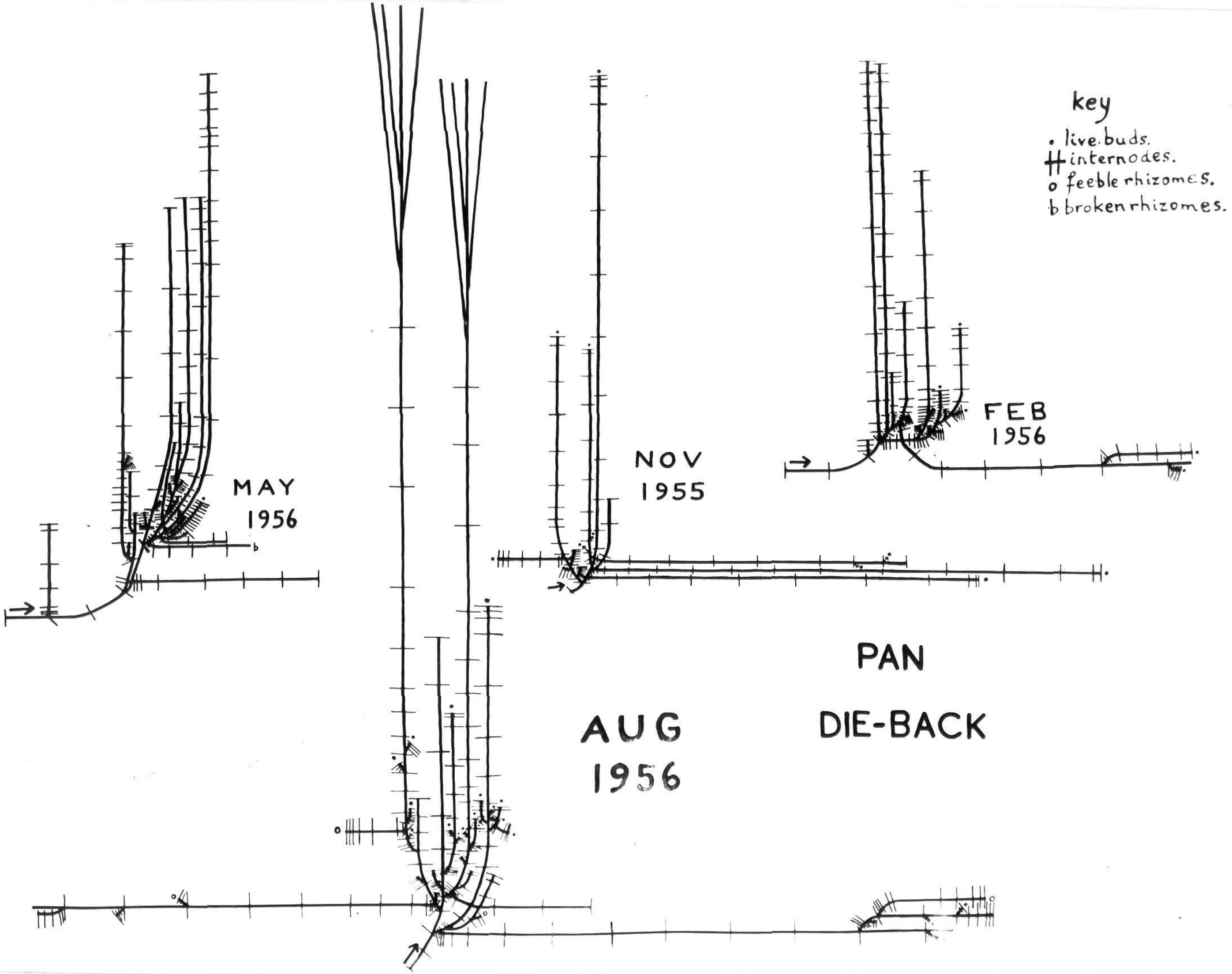


FIG. 19. Lymington pan "die-back" plants in May, August, November and February. Note poor survival in May; long rhizomes; slight activity in August; bud deaths and long rhizomes, with dead or feeble apices, in November.

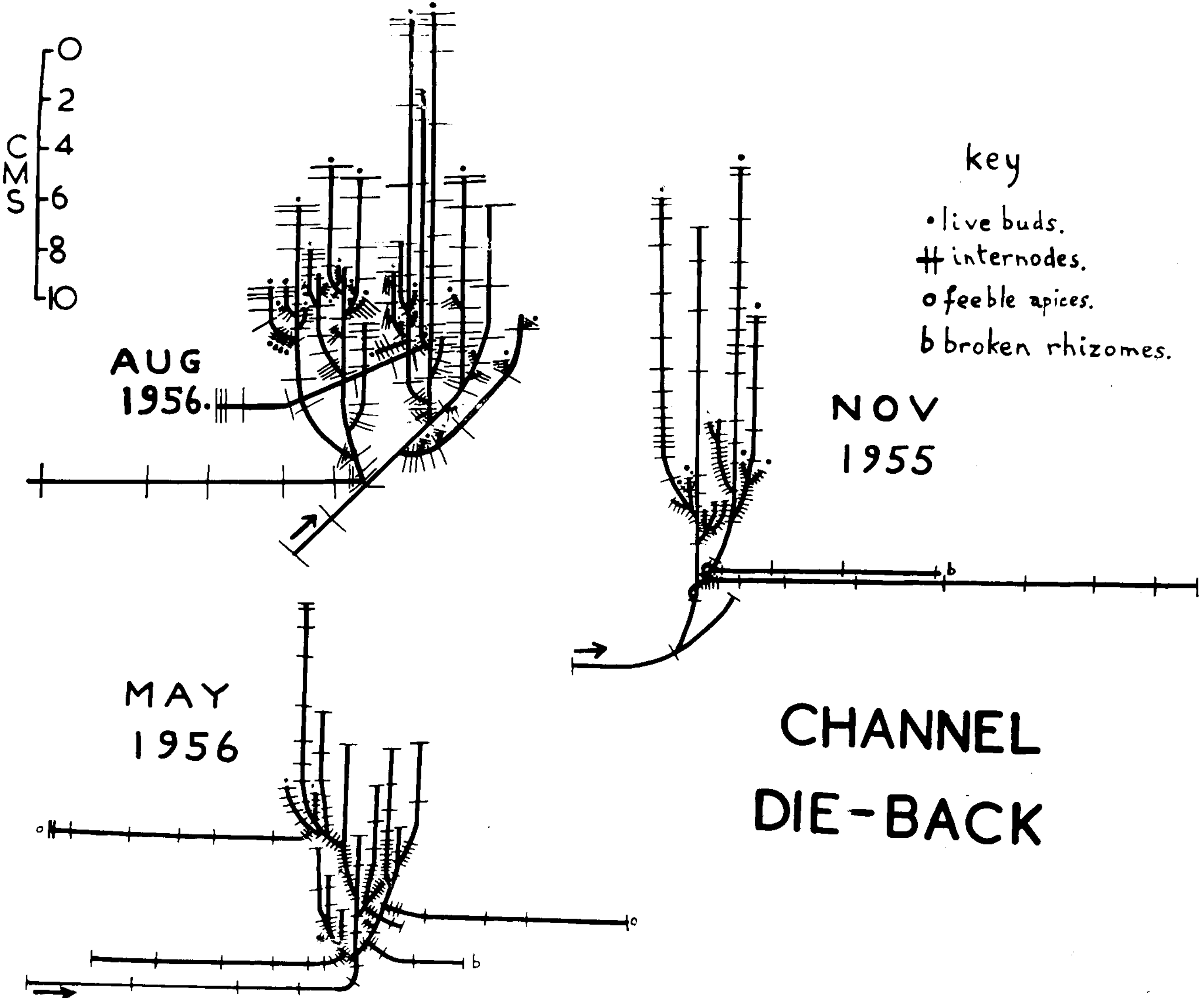


FIG. 20. Lymington channel "die-back" plants in May, August and November. Note poor survival in May; long rhizomes; slight activity in August; bud deaths and long rhizomes, with dead or feeble apices, in November.

Throughout the early summer the plants of the "die-back" areas were most variable. By August some appeared to be quite active, with flowering culms of 50 - 55 cm. (rather less than in healthy sward). These had as many as 14 leaves, as opposed to the normal 8 - 10 in healthy sward, suggesting that flowering had been delayed. Short stems, of 6 - 15 cm., represented the second generation of tillers. The buds, which would provide the third generation of tillers, had already suffered 50% deaths by August (Figs. 19, 20).

Despite the reduced vigour of the plants, they appeared, in August, to be healthier than at any other time of the year. It is believed that, over winter, in "die-back" conditions, the weakest plants die, and consequently a thinner but healthier sward carries on through the summer.

By November some of the old, tall stems in the "die-back" plants had flowered. Of the shorter, later flowering second and third generation tillers, 75 - 100% were dead. The buds, which should have provided the bulk of the following year's flowering stems, developed scantily, usually an average of only one forming on each stem, where three might form in healthy sward. In November, too, there were very long rhizomes which had apparently remained underground during the summer, instead of turning up to produce new rosettes in the spring, as happened in the healthy sward. These rhizomes in the "die-back" plants in November had dead or feeble apices, like those found in May. Sometimes even some of the lateral buds replacing the rhizome apices had died (Figs. 19, 20).

A large unit of Spartina was dissected from the channel "die-back" edge in February 1955. It consisted of many shoot clusters of the type previously examined, each cluster joined to the one ahead by a rhizome. The clusters consisted of from ten to thirty stems, of which from one to three were alive (in the last cluster, three of six were alive). The live stems were only 5 - 10 cm. tall, and were over-wintering, being the last generation of tillers from the previous year. (Fig. 21).

Some of the stems in the clusters were "waisted", appearing thinner in the middle of the culm than above or below, and this was found to be associated with the occurrence of several short internodes in mid-culm (w in Fig. 21). Normally the longest internodes of the vegetative culm appear in the middle (Jacquet, J., 1949), but in waisted stems there is a region of longer internodes both above and below the middle, showing that stem elongation has been checked at some time, as suggested previously to explain the high leaf numbers on flowering stems. Waisting was a rather common condition in "die-back" plants (Figs. 19, 20, 21).

In the February collection there was an almost complete lack of the buds which, formed in the previous

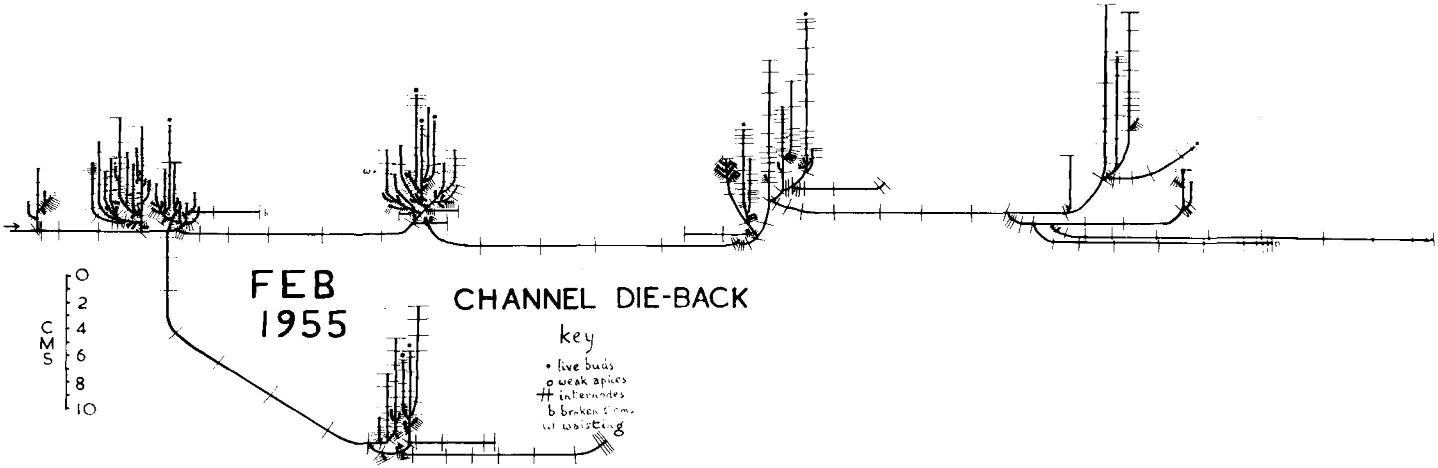


FIG. 21. Lymington channel "die-back" plant in February. Note moribund clusters of stems, some waisted (w); also high bud deaths, and feeble rhizomes.

winter, should have provided the bulk of the flowering stems for the year. 80 - 95% had died. This must have resulted in a great reduction of the flowering which should have taken place.

There had been few successful rhizomes, the two last formed being feeble, while only one usually developed from each shoot cluster, where several were found in healthy plants. Rhizomes running between the shoot clusters of the February plant were almost horizontal, suggesting that very little accretion was occurring in the area.

In "die-back" areas Spartina appears to be making no progress, barely maintaining itself by subnormal growth during the summer, and suffering degeneration during the winter, on virtually static substrates.

(iii) Torn and dwarfed plants.

Plants from the area on the east bank of the Lymington estuary mouth, near No. 5 Post, at MR 34509405, which were torn and dwarfed, showed a very different pattern of growth over the preceding year from that of plants in the "die-back" areas upstream.

The area was inaccessible at certain times of the year when heavy seas were running, as it was approachable only from the sea, and observations were consequently fewer.

In February, though strong and dark green, the upright stems were dwarfed, being only 1 - 2 cm. tall. 90% were dead, and the form of the plants was unusual. Though strong and wiry, the stems were abnormally thin (0.2 - 0.4 mm., as opposed to healthy plants of 0.3 - 0.6 mm. diam.) The internodes were exceptionally short, less than 1 mm. in many cases. The proportion of buds alive in February (25%) was lower than in healthy plants (85%), but rather higher than in "die-back" plants (5 - 20%). The rhizomes were like the stems in having extremely short internodes. Like the healthy plants, plants of this area had upwardly growing rhizomes, showing that accretion was occurring quite rapidly (Fig. 22).

In August the plants of this area, like those of the "die-back" areas, were looking healthier than at any other time of the year. 60% of the stems were alive, and flowering was occurring vigorously - but on culms of only 20 cm. height, the shortest recorded, yet having 8 - 10 leaves. The inflorescences had only two spikes, and these were short. The number of buds at this time was, however, 2 - 3 per stem on average, a similar number to that found in the healthy sward, and better than that found in the "die-back" areas (Fig. 22).

The sward in this area appeared far more densely populated at this time than in the healthy site, and there was apparently no lack of short stems produced from the buds. The loss of stem material in this site did not occur at the bud stage, as in the "die-back" areas, but later, when the stems had developed.

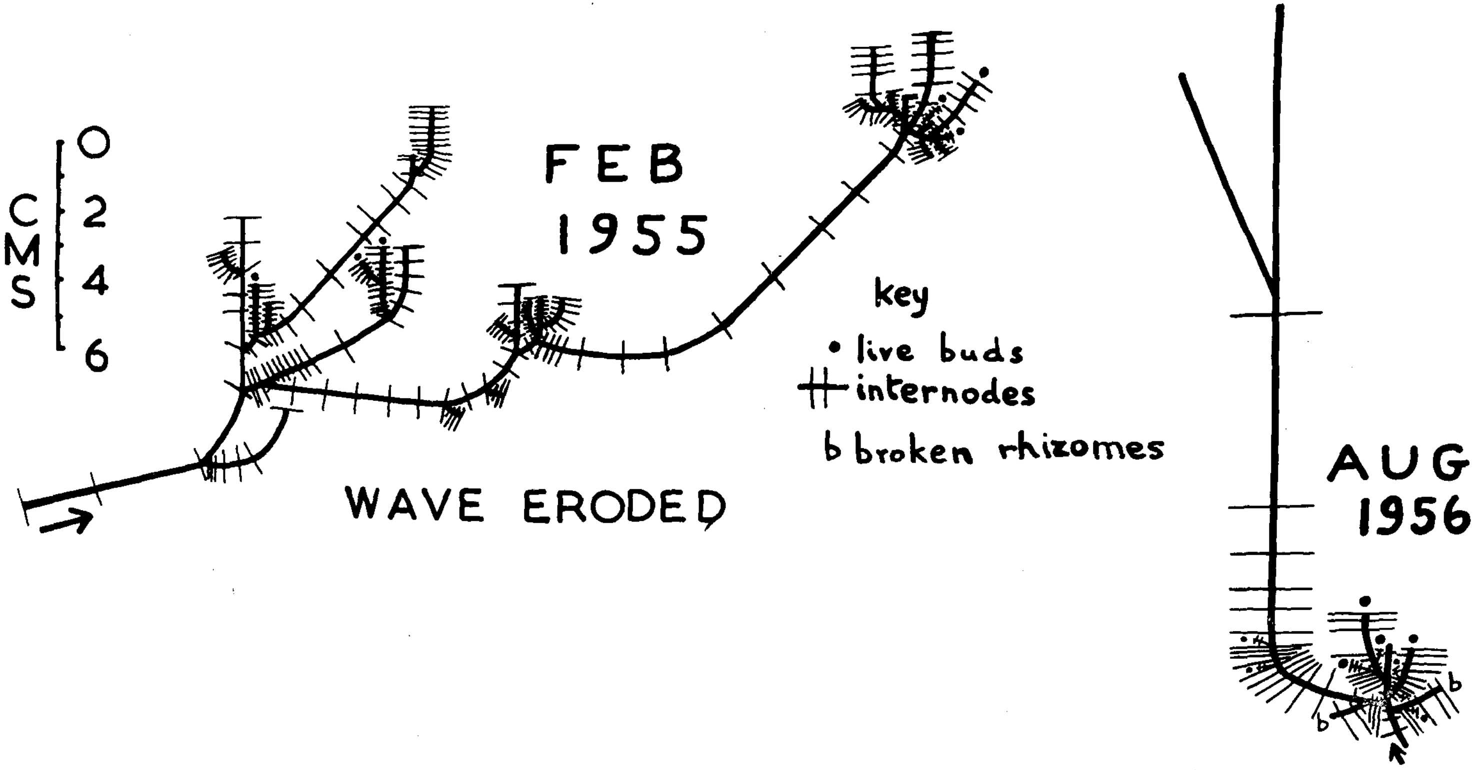


FIG. 22. Lymington torn and dwarfed plants in February and August. Note extreme shortening of internodes - and small inflorescence, with slightly higher bud numbers than in "die-back".

If the eroding action of the wind and waves was, in fact, the cause of the dwarfing and destruction of the sward in this area, it would be expected that whole stems would be removed rather than a reduction in bud numbers occur, as in the "die-back" sites. Removal of stems would have the double effect of stimulating bud production by loss of upright apices, and reduction of stem height by cutting down leaf numbers. Loss of leaves would undoubtedly reduce synthesis, and cause depauperation.

Both the expectation of bud stimulation and that of dwarfing are borne out in this site, and there seems little doubt that the dwarfing of plants from the mouth of the Lymington river, and upstream on the east bank as far as the Tar Barrel (MR 34139433) is caused by wave action.

In eroding conditions it seems that vigorous response to the environmental circumstances is induced in the Spartina, and vegetative reproductive activity is increased, but the loss of material may be such that the plant is hardly able to grow sufficiently to maintain itself.

(c) Conclusions.

Study of the germination and growth of Spartina was made possible by low temperature moist chamber storage of seed, followed by fresh water sand culture in the temperate greenhouse. The rate of leaf production varied with temperature, but was unaffected by either cold treatment of the seed or daylength variation during growth. In the temperate greenhouse the plastochrone was around 2½ weeks. Outdoors, it was about 4 weeks. Leaf production ceased on flowering. Thus, up to the time of flowering, leaf number was a measure of the age of the plant, and the temperature at which it had been grown. By contrast, main stem growth was very sensitive to daylength treatment, growth being greater in long days than in short days. Flowering only occurred in long days.

Vegetative reproduction took place by growth of buds in the axils of the leaves of the main stem. Buds developed either as rhizomes or as tillers, depending to some extent on their position. More buds developed in short days than in long days. Vegetative reproduction was most effectively brought about by rhizome production and outward and upward growth to produce new stems at a distance from the parent.

Using the observations on development, it was possible to interpret the morphology of the Spartina plants in the field. On this basis, the radial development of tussocks and their cyclical behaviour, giving rise to fairy rings, were explained by Mrs. P-A. Caldwell.

Morphological examination of plants from Lymington showed that in healthy sward they had rosette form in May, with many buds developing in the leaf axils. Rapid elongation of the culms took place during the long days of summer, till they rose to a height of up to 80 cm. Flowering was vigorous, and as the short days of winter set in, many buds were laid down, 85% of which survived until the spring.

"Die-back" plants were yellowish and unhealthy looking during the winter, with fewer rosettes forming in the spring than in the healthy sward. Of the buds on the rosettes, 80 - 95% had died during the winter. Long feeble rhizomes persisted. Although flowering occurred, it was not vigorous, and the culms rose only to a height of 55 cm. Death of buds and failure of rhizomes occurred throughout the year. Overwintering stems were frequently waisted, with large numbers of short internodes, showing that they had grown for long periods before flowering.

Torn and dwarfed plants from the area of the river mouth had rosette form in the spring, with 75% bud deaths. Internodes of the stems and rhizomes were extremely short. Flowering occurred in the summer on culms only 20 cm. tall. The dense growth on the ground, however, suggested that some kind of stimulation was occurring to produce excessive budding. This appeared consistent with the probable effects of wave battering.

Morphological examination of plants had thus emphasised the strong growth in healthy sward, the bud death in "die-back" conditions, and the dwarfing and damaging at the river mouth. It was decided to carry out quantitative analyses of the sward to determine the importance of the processes observed, and, by simple field trials, to attempt to reproduce them.

(3) Field trials - Sward sampling.

(a) Scope.

In support of the morphological observations, seasonal activity and productivity were measured in the field. The areas in which the study was carried out were similar to those from which plants had been taken for morphological analysis. These were:-

(i) The area of healthy sward, MR 340946.

(ii) The area of dwarfed sward near the river mouth, MR 345941.

(iii) The area of channel "die-back" near the Pier, MR 334954.

(iv) An area of pan "die-back" near No. 11 Post, MR 340950.

The area of pan "die-back" studied here differed from that used in the morphological studies as this proved too small for sward analysis, and another similar, but larger, area had to be selected.

The study of the untreated areas of sward was supplemented by a study of similar areas which were cut to ground level, to determine whether the cutting action of the waves was sufficient to account for the dwarfing and bud stimulation at the river mouth, and to discover whether similar bud stimulation could be effected in "die-back" areas, by artificial cutting.

Quadrats were laid down and sampled in the selected sites. At two monthly intervals shoot weights were taken, and total shoot numbers and flowering head numbers counted, for both live and dead plants, sothat the turnover of material could be determined. Histograms of heights and leaf numbers of live shoots were prepared, and average values calculated. Shoot height had already been shown to vary with daylength, but it also reflects the vigour of the plants. Leaf number is principally a reflection of the age of the shoot, though it also depends on the temperature at which the plant has been grown (see Section IV, 1). Short shoots and high leaf numbers were also characteristic of unhealthy "waisted" plants. Samples were also taken from below the sward to determine the underground activity and turnover of live and dead material. Especial attention was paid to the detection of rhizome failure.

(b) Methods.

Plants in the sward were treated, for the purposes of sampling, as being composed of above and below ground parts. Ground level was, in the virtually static sward at Lymington, a near approximation to the centre of the rosette region of short internodes separating the upright shoots from the roots and rhizomes.

(i) Above ground samples.

Above ground samples, consisting of the main shoots and their tillers, were taken by marking out metre quadrats and sampling in these larger quadrats, smaller quadrats of 1,000 sq.cm. (25 cm. x 40 cm.) at two monthly intervals. Sample 1,000 sq.cm. quadrats were taken from the south-east corners of the large quadrats, then progressively at 25 cm. intervals northwards, until reaching the end of the large quadrat. Then northwards along a line 40 cm. west of the first, and so on, in parallel ladder fashion.

Sampling of the 1,000 sq.cm. quadrats was carried out by cutting all the stems and tillers at ground level with a sharp knife and removing them to the laboratory for analysis. The shoots were sorted, dead ones being counted and weighed. Live shoots, in addition to being counted and weighed, were measured from the cut end to the tip of the last, upright leaf, and the number of emerged leaves above the rosette counted. The number of flowering shoots was also noted. The large number of measurements required decided against measurements of the true stem heights (which are less than shoot heights by the length of the last leaf), and internode numbers (which are greater than the leaf numbers by the number of unemerged leaves), in favour of the more easily observed shoot heights and leaf numbers.

(ii) Below ground samples.

Below ground samples were taken by placing a tin of 8 cm. diameter and 10 cm. depth, open at both ends, on one end in the centre of the 1,000 sq.cm. quadrat which had been sampled, and pushing the tin into the soil while cutting round it with a knife. When it was sunk to the top it was dug out, with the cylinder of soil inside.

The soil was then washed away from the plant material, in a sieve, and the plant parts divided into live and dead rosettes, rhizomes, absorbing roots, intermediate roots, and holding roots, to test quantitatively the morphological effect of each treatment on the below ground parts. The samples were left to air dry, and weighed. It was later discovered that variations in the weights of the individual parts were too large between comparable samples to allow any general interpretation of the results, and accordingly the weights were added to give the total live weights and dead weights of the below ground parts. These totals were found to give some indication of the overall underground activity of the sward, and were more consistent than the individual part totals, previously discarded. The appearance of a sward sample undergoing analysis is shown in Fig. 23. The method of sampling was developed in conjunction with Miss E. M. Uren.

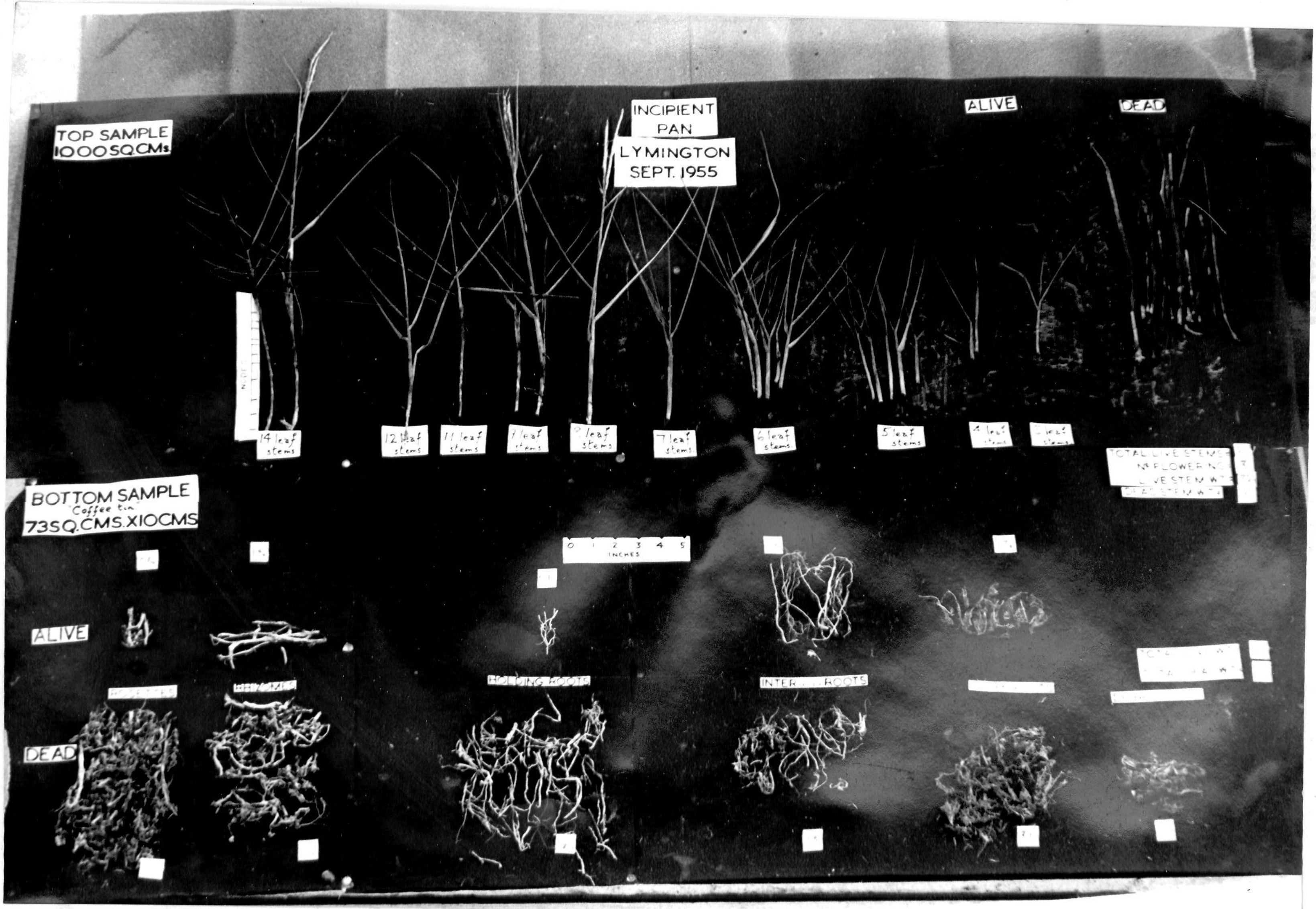


FIG. 23. Lymington sward sample undergoing analysis. Note the thin growth and waisted stems, in this 1000 cm. quadrat.

(c) Results.

The results of the sward analyses are given in full in Appendix 3, but their implications are considered below:-

(i) Shoot numbers, shoot weights, and flowering head numbers.

α) Seasonal trends.

The lowest live shoot numbers (13 - 45/1,000 sq.cm.) and average shoot weights (0.02 - 0.7g./shoot) were reached in all sites in March, when all the plants of the sward had rosette form. At the same time, dead shoot weights (6 - 74g.) and numbers (22 - 84) were at or near their maximum, all the flowering culms of the previous season having matured and died. The lowest dead shoot weights were found in the dwarfed site, and this was accountable by the removal of dead stems by wave action, as well as their small stature.

The shoot numbers in "die-back" sites reached their maxima in May (47 - 71), while that in the dwarfed site was a maximum in August (264), and that in the healthy site was a maximum in September (63). The early and unmaintained maxima of live shoot numbers in "die-back" sites were undoubtedly due to the loss of buds during the winter. Flowering maxima were reached in all sites in August and September.

In autumn shoot numbers were minimal, falling to 152 in the dwarfed site, 56 - 66 in the healthy site, and

23 - 42 in the "die-back" sites.

β) Site differences.

The most striking difference was between the dwarfed site and the others. Except in March, the dwarfed site never had fewer than twice as many shoots as the others. Both live and dead shoots were short and light in weight in the dwarfed site. Flowering was by far the most vigorous in the dwarfed site (45 inflorescences being collected in the year's samples).

The healthy sward had higher live and dead shoot weights and flowering head numbers (20/year) than the "die-back" sites, where flowering numbers were only 5-9/year, suggesting that the productivity and turnover of material is much more rapid in the healthy site than in the "die-back" sites.

The pan and channel "die-back" sites varied rather little between themselves, though in the pan "die-back" conditions the live shoot weights were generally higher than in the channel "die-back" conditions, as were the dead shoot weights and numbers. Flowering was slightly more vigorous in the pan "die-back" site than in the channel "die-back" site. These facts show that the pan "die-back" area chosen in this study had a slightly great productivity and turnover of material than the channel "die-back" site.

(ii) Shoot heights and leaf numbers.

α) Seasonal trends.

Histograms of the live shoot heights and leaf numbers were plotted for September and March samples, those differing most widely, in all sites (Fig. 24). The greatest variation was in mode and range but nevertheless the differences were sufficiently great for the use of mean values without statistical analyses. Average shoot height and leaf number were at a minimum in March in all sites, being most noticeably low in the dwarfed site (3.6cm., 2.7 leaves) less so in the "die-back" sites (6.0 - 10.5cm., 3.0 - 4.7 leaves), and least of all in the healthy site (24.0cm., 5.3 leaves). In May, with the new flush of buds, the healthy site had a lower average shoot height and leaf number (12.6cm., 2.9 leaves) than the "die-back" sites (13.7 - 16.0cm., 3.3 - 4.0 leaves).

By August maximum average shoot heights had been reached in dwarfed sites (14.0 cm.) and "die-back" sites (23.4 - 26.0 cm.). The healthy site reached its maximum average shoot height in September (44.7 cm.). After September, though the average shoot heights had stopped rising, average leaf numbers showed a steady increase until November.

β) Site differences.

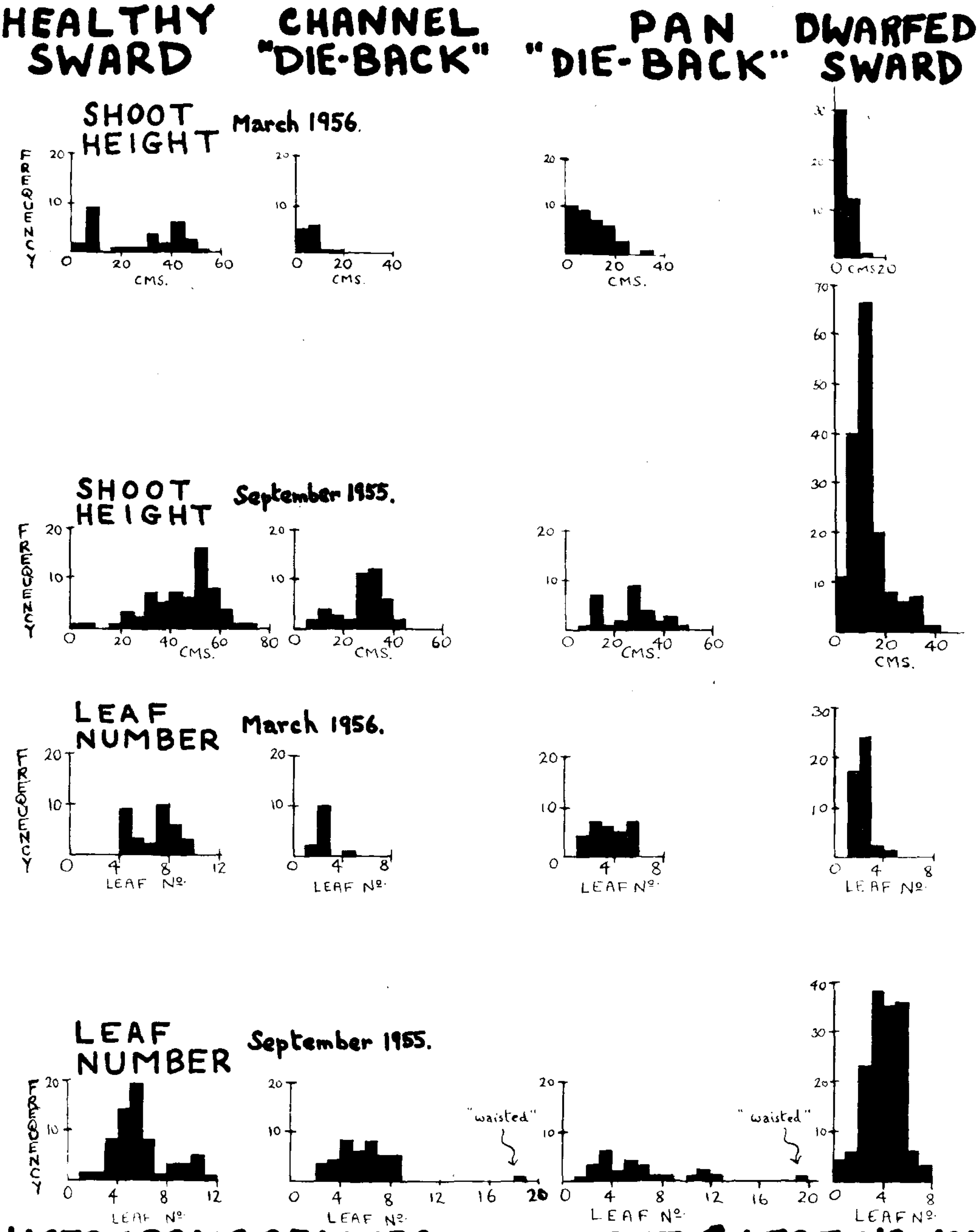
Striking differences were apparent between the histograms of the live shoot heights and leaf numbers of the dwarfed site and the others (Fig. 24). In every collection the average shoot heights and leaf numbers were much lower in the dwarfed site than in the healthy or "die-back" sites. For most of the year the shoots in the dwarfed site were on average less than half the average height of the shoots in the healthy site. The low leaf numbers show that there was a vigorous young bud population in the dwarfed site.

The "die-back" shoot heights were, on average, lower than the healthy shoot heights, except in May. The two "die-back" sites differed little in average shoot heights. Average leaf numbers varied scarcely at all between the healthy and "die-back" sites. Presumably this was mainly a reflection of age, without the complication of removal of shoots which undoubtedly altered the average shoot heights in the dwarfed site.

(iii) Below ground samples.

α) Seasonal trends.

The most noticeable trend in the below ground samples was in the healthy site, where the large amount of live material underground showed a steady increase from September until January. This reflected the growth of rhizomes below ground. Other sites showed the same tendency to high live weight below ground in the winter months, but not as markedly. There was in all sites less live material below ground in May - August, and this was a



HISTOGRAMS OF LIVE SHOOT HEIGHT & LEAF No. IN MARCH & SEPTEMBER.

FIG. 24. Histograms of live shoot heights and leaf numbers for September and March samples, in healthy sward, channel "die-back", pan "die-back" and dwarfed sites. Note taller stems in healthy sward, higher "waisted" internode numbers in "die-back", and extreme dwarfing in eroded site.

consequence of the upturning of the rhizome tips, their establishment, and death of the rhizome behind them, causing a maximum dead weight in September especially noticeable in the healthy site. Variation in dead weight was scarcely noticeable in "die-back" sites. Continual rhizome failure and death probably maintained dead weight at a constant high level.

β) Site differences.

The marked seasonal activity seen in the healthy site did not occur in the other sites. Productivity and turnover were greatest in the healthy site. In the lack of vigour below ground the dwarfed site found its only resemblance to the "die-back" sites.

(d) Summary - i. Seasonal trends.

Sward analyses showed that there was a marked rhythm of seasonal activity in Spartina. In March the sward consisted of rosettes with few leaves, and undeveloped tiller buds. Interspersed with these were the persistent dead culms from the previous autumn's flowering. Below ground were the dead parts of rhizomes whose growth had terminated with the formation of rosettes at the soil surface.

Spring growth began in May, when "die-back" sites were quite active. Dwarfed and healthy sites, however, were still producing buds at this time. These buds led to vigorous and sustained activity above ground and flowering in August and September, when there was almost no live material below ground.

After September shoot growth slowed, though there was a steady increase in the average leaf number through the winter months. At the beginning of the winter, however, bud formation began, and the winter months were chiefly characterized by a steady increase in live material below ground. This rhizome activity was most marked in the healthy site. In other sites it was masked by continual rhizome failure and death, giving high dead weights below ground.

ii. Site differences.

The extremely high shoot numbers and flowering head numbers in the dwarfed site - together with the very small shoot heights and leaf numbers - made it strikingly different from all the other sites.

"Die-back" sites were thinly populated with shoots, which were rather short and whose buds failed, with the result that activity was confined to a short period in early summer. There was continual rhizome failure throughout the winter months.

Healthy sites had a vigorous turnover of material and a high productivity, producing tall stems in the summer months and strong rhizomes in the winter months.

The sward analysis emphasised the differences between "die-back" causing bud failure and death, and dwarfing and bud stimulation associated with wave erosion. This latter phenomenon - especially bud stimulation and its possible application to "die-back" sites - was made the subject of an experimental study.

(4) Field trials - Experimental cutting.

(a) Methods.

Exactly similar methods were used in laying out quadrats in this experiment as in the previous sward sampling experiment. The quadrats used in this experiment were adjacent to the untreated quadrats in the healthy area, the pan "die-back" area, and the channel "die-back" area. The quadrats for cutting were first sampled before treatment, in September 1955, and then all the shoots were cut at ground level. Sampling was then carried out at two monthly intervals as in the previous experiment, until August 1956.

(b) Results.

(i) Shoot numbers, shoot weights, and flowering head numbers.

The number of shoots increased as the time from cutting progressed. The live shoot weight increased rather more slowly. No dead shoots were found during the experiment, all the new shoots remaining alive for the year of the experiment. A maximum number of live shoots was reached in May - August after cutting in September. "Die-back" sites produced only 21 - 37 shoots and no flowers, but the healthy site grew 116 shoots, and flowered vigorously. There had obviously been strong bud stimulation in the healthy site.

(ii) Shoot height and leaf number.

The average shoot heights increased during the experiment, returning to their former levels in the "die-back" sites by August. In the healthy sward the average shoot height remained low, and the average leaf number exceptionally low (1.8), in a manner resembling the dwarfed sward.

(iii) Below ground samples.

The live weights below ground were high in autumn and showed seasonal activity only in the healthy sward. As in the untreated quadrats, productivity and turnover were far greater in the healthy site than in the "die-back" sites.

(iv) Summary.

The cutting treatment only markedly affected the healthy site. The bud population was stimulated, and a dense growth of short shoots was produced, followed by strong flowering. No such stimulation occurred in the "die-back" sites, where the bud failure is clearly of a fundamental nature.

The resemblance between the healthy site after cutting and the dwarfed site was remarkable. Both had the same dense growth of short shoots, and there can be little doubt that the dwarfing which occurs at the river mouth is due to the cutting action of the waves in that area.

D. Conclusions.

The morphological survey of Spartina growth outlined the processes of building and maintenance of the sward. Examination of plants and sward in the areas selected during the ecological survey at Lymington showed that healthy sward was characterized by strong growth above ground in the summer and by bud development and vigorous rhizoming below ground in the winter months.

Dwarfing and bud stimulation followed removal of stems from the sward in otherwise healthy areas. There can be little doubt that wave action in the area east of the river mouth caused the dwarfing and high shoot density in the sward at that point.

"Die-back" sward was characterized by failure in the production and survival of buds during the winter, and death of rhizomes. No stimulation of bud production could be achieved by cutting in "die-back" sites. The factor controlling bud failure was clearly a fundamental one, and seemed likely to be part of the habitat complex.

V. MINERAL NUTRITION.

A. Introduction.

"Die-back" of Spartina showing, as it does, certain physiological features, such as the rhizome failure and the altered morphology of the plants, might be caused by mineral unbalance in the soil. A study of the mineral nutrition of Spartina was carried out in the field and in the laboratory.

B. Field trials - Experimental fertilizing.

1. Materials.

A first approach to the problem of mineral unbalance in Spartina was made by spreading an impure inorganic fertilizer on the sward, and carrying out morphological analyses of the plants over the following year. Inorganic fertilizer was chosen as being unlikely to interfere greatly with soil micro-organism balance, yet likely to provide minor plant elements, should they be deficient. For this purpose Fisons' No. 1 fertilizer, with an advertised rating of "7 nitrate, 7 phosphate, and 10½ potash", was used at the rate of 4 ozs. to the square metre, a rate considerably in excess of that recommended for lawn grasses.

2. Methods.

Quadrats similar to those used for the untreated and cutting trials and subsequent sward analyses in Section IV above, but slightly removed from them to avoid contamination, were analysed in September 1955, and then broadcast with fertilizer. Sampling was carried out at two monthly intervals until August 1956.

3. Results.

Fertilizing had no effect on bud numbers in "die-back" sites. There was a slight apparent increase in the bulk of the individual stems, though there was no increase in numbers. There was neither increase in stem weight nor in stem numbers in the healthy site, after fertilizing. The increase in stem weight in the "die-back" areas is not great, and is not likely to be of importance, and certainly shows no amelioration of "die-back" conditions. There was no improvement of the growth below ground after fertilizing.

A similar experiment in which the stems were cut at the same time as being fertilized, likewise showed no improvement in the "die-back" conditions. In this case after cutting, a blanket of algae formed over the healthy sward quadrat, which then produced no new shoots.

4. Discussion.

It is evident that application of fertilizer to the

Spartina sward causes little change and effects no improvement in "die-back" areas. Bearing this in mind, mineral deficiency seemed unlikely, in "die-back" areas. There was still the possibility, however, that some element might not be available to the plant, and this could only be detected by carrying out a detailed laboratory investigation.

C. Mineral analyses.

An important technique for investigating the possibility of a mineral deficiency - applicable to both short supply and non-availability - is analysis of plants and soils in connection with the known clinical symptoms (Goodall, D.W., and Gregory, F.G., 1947). This technique was employed in the investigation on Spartina.

1. Materials.

(a) Plants.

Similar morphological units of representative Spartina plants were collected on April 19th, 1955 from the areas at Lymington which had been studied ecologically and morphologically. The areas were:-

i) The healthy sward at Cocked Hat in mid-estuary MR 33999464.

ii) The channel "die-back" edge near the Pier, MR 33409535.

iii) The pan "die-back" edge near the Pier, MR 33409540.

(b) Soils.

At the same time as the collection of plants was made, samples of the top 5 cm. of the soils in which the plants were growing were taken for analysis.

2. Methods.

(a) Plants.

i) Major elements.

α) Cleaning.

The whole plants were quickly but thoroughly cleaned by washing in running water, and all the oldest leaves, in which the soil was too deeply engrained to be removed by washing, were stripped off.

β) Drying.

The plants were blotted dry with filter papers, cut at rosette level into above and below ground parts, and then separately weighed, and quickly dried. Dry weights were taken, and the samples were cut small to reduce the fibre length; ground with a pestle and mortar; and stored in well stoppered bottles. Cutting and grinding dry material were found to be easier, and loss by exudation was less, than when fresh material was used.

γ) Ashing and silica determination.

Ashing was carried out by the dry method of Piper (1950). Rough silica estimations were made by leaching the ash in 50% HCl overnight, igniting the residue, and reweighing. Silica free ash weights were obtained by difference.

δ) Total nitrogen determination.

Since no good sample could be obtained from fresh material, the long, coarse fibres withstanding all attempts at grinding, nitrogen determinations had to be carried out on dried material, carefully quartered, and taken in 0.1 g. samples. Determinations were made using a Markham still in a technique similar to that of Humphries (1956). Mean values were calculated for quadruplicate determinations. Individual determinations varied from the mean values by not more than 2% in this and in subsequent chemical determinations.

ε) Total phosphorus determination.

Replicate digestions were carried out by the method of Gerritz (1935), using nitric and perchloric acids. Determinations of the phosphorus in solution were made by using the ammonium vanadate reagent of Barton (1948), producing a yellow colour, which was measured in a Hilger and Watts Spekker, with a blue filter, No. 1.

϶) Alkali metal determinations.

Digestions were carried out by standing replicate fresh, rough ground 0.25 g. samples in 1 ml. of A.R. nitric acid in 5 ml. beakers, for one hour: diluting to 2 ml., and leaving overnight: then washing the solution quantitatively through a filter paper into a 100 ml. graduated flask until reaching the mark. The solutions were then used for determinations of sodium, potassium, and calcium.

Sodium and potassium determinations were made with the Evans Electroselenium Limited "Eel" Flame Photometer, using the standard methods recommended in the makers' "data sheets" Nos. D5 and D6.

Calcium determinations were preceded by the following separation for Na, K, and Mg, which interfere with the calcium flame determination:-

To 20 ml. aliquots of the digest for alkali metals was added 1 ml. M/10 A.R. oxalic acid solution, then M/10 A.R. ammonium oxalate solution, until the solution was pH 4 - 5, (about 50 ml. needed). At pH 4 - 5 magnesium oxalate remained in solution, but calcium oxalate was precipitated. The precipitate was allowed to settle, and washed by decantation until no Na, K, or Mg remained. The precipitate was then dissolved in 0.05 N perchloric acid solution, and this solution was used in the flame photometer to measure calcium by the standard method (Powell, F.J.N., 1953).

All determinations for alkali metals required, for dilutions, distilled water which was free from Na and K. Calcium determinations required distilled water free from Na, K, Mg, and Ca. Glass distilled water was used, and checked with the flame photometer for absence of these elements.

η) Chloride determination.

Chlorides were determined on 5 ml. aliquots of the digest for alkali metals; neutralized with chloride-free calcium carbonate, and titrated with N/100 silver nitrate solution, using potassium chromate as indicator.

(ii) Minor elements.

α) Cleaning and drying.

The outer parts of plants for determination of minor elements were washed in glass distilled water to remove as much soil as possible. All the outer parts were then removed from the shoots, leaving only the youngest leaves, and the stems. These above ground parts were then dried in thoroughly cleaned beakers. After the roots and rhizomes had been cleaned as thoroughly as possible in glass distilled water, these, too, were dried, separately. The dry material was cut to smaller size with scissors, using them no more than was absolutely necessary, to avoid contamination.

β) Determinations.

Spectrographic determinations of the minor elements Copper, Manganese, Molybdenum, Cobalt, Nickel, Chromium, Vanadium, Lead, Tin, and Zinc were kindly made by Mr. H.H. LeRiche, of Rothamsted.

(b) Soils.

(i) Major elements.

α) Calcium carbonate determination.

After drying the soil in the oven at 90°C., a determination of calcium carbonate was carried out by the "rapid titration" method of Piper (1950).

β) Total nitrogen determination.

Digestion and determination of nitrogen were carried out on the dry soil samples by the same methods as were used in plant analyses.

γ) Calcium determination.

Calcium was determined by the same methods of digestion and measurement as were used in the analysis of plant samples.

δ) Determination of other elements.

Sodium, potassium, chloride, and "available" phosphorus were determined on the solution after citric acid treatment of the soil. 10 g. of the dry soil were left standing in 100 ml. of water, containing 1 g. of citric acid, for one week, in accordance with standard techniques. The resulting solutions were used in direct determinations of the elements by the same methods as in the plant analyses.

(ii) Minor elements.

It is known that minor plant nutrients, though present in the soil in quantity, are often not in an available state.

There are no completely general techniques which are easily applicable, and reliance is thus usually placed on the plant analyses of minor elements to show differences in availability. Simple qualitative tests were employed in this investigation for the presence of iron and manganese only.

3. Results and discussion.

(a) Major elements.

(i) Plants.

α) Results.

The results of analyses of plants for the major elements are summarised in Table VII. For comparison the results of the only other determination of the mineral content of *Spartina*, carried out by Knowles (1929) on material from Poole Harbour, are added.

β) Discussion.

There were no great differences between the level of any mineral in the healthy site, and the levels in the "die-back" sites. The greatest difference was in the nitrogen level, which was nearly twice as high in the healthy roots and rhizomes as in those of "die-back" plants. This, however, seemed more likely to be a reflection of the poor state of the plants than a severe nitrogen deficiency.

TABLE VII.

Analysis.	Site of collection and part of plant.						
	Poole 1929	Lymington 1955					
	Shoots & leaves.	Healthy shoots & leaves.	Healthy roots & rhizomes.	Channel "die-back" shoots & leaves.	Channel "die-back" roots & rhizomes.	Pan "die-back" shoots & leaves.	Pan "die-back" roots & rhizomes.
A. MOISTURE							
Moisture content.	77.9%	75.66%	81.14%	83.14%	86.73%	80.39%	80.80%
Dry weight	22.1%	24.34%	18.86%	16.86%	13.27%	19.61%	19.20%
B. CHEMICAL ANALYSIS (dry wt. basis)							
Total Nitrogen	-	1.87%	2.33%	1.67%	1.55%	1.56%	1.61%
Silica free ash	8.69%	12.56%	9.60%	9.79%	13.79%	12.61%	11.78%
Phosphoric acid	0.78%	0.48%	0.48%	0.55%	0.58%	0.47%	0.53%
Potassium	1.82%	1.26%	0.98%	0.68%	1.25%	0.73%	1.65%
Sodium	-	3.37%	1.81%	1.71%	1.11%	2.63%	1.81%
Calcium	0.45%	0.31%	0.16%	0.33%	0.24%	0.36%	0.10%
Chlorine	2.72%	3.75%	2.30%	2.10%	0.90%	3.00%	1.85%

Other minor variations included those in the potassium levels, which, in the healthy plants were higher in the shoots, but in the "die-back" plants were higher in the roots. It may be that potassium is transported upwards to the active apices of the young shoots in the healthy plants, but in the "die-back" plants no such "sinks" for potassium are available, and most of the potassium stays in the lower parts of the plants. The existence of "sinks" has been shown in barley on feeding with phosphate (Russell, R. Scott, 1954).

The elements potassium, sodium, and chlorine were all present in slightly greater quantity in the shoots and leaves than in the roots and rhizomes of plants in all sites. This further supports the theory of the existence of "sinks" of varying strengths, in the upper parts of the plants. Sodium chloride is almost certainly taken up in considerable quantity by Spartina, and Skelding and Winterbotham (1939) have suggested that it is eliminated from the leaves. In the intertidal periods salt crystals do seem to form on the leaves, and the elimination of these may be associated with high salt concentration until the next tide.

There were no great differences between the results obtained by Knowles (1929) in the healthy sward at Poole and the results obtained in the recent analyses, and it is concluded that, on the major mineral content of the plants, mineral deficiency seems unlikely.

(ii) Soils.

α) Results.

The results of the chemical determinations of the major mineral plant nutrients in the soil were as follows (Table VIII):-

TABLE VIII.

Analysis.	Healthy sward.	Channel "die-back".	Pan "die-back".
"Calcium carbonate"	trace	trace	trace
Total Nitrogen	fw = 0.003% dw = 0.0048%	fw = 0.10% dw = 0.43%	fw = 0.13% dw = 0.54%
Available Phosphorus	fw = 0.006% dw = 0.0087%	fw = 0.006% dw = 0.026%	fw = 0.005% dw = 0.021%
Potassium	fw = 0.0004% dw = 0.0006%	fw = 0.0004% dw = 0.0020%	fw = 0.0006% dw = 0.0024%
Calcium	fw = 0.05% dw = 0.08%	fw = 0.05% dw = 0.22%	fw = 0.08% dw = 0.32%
Sodium chloride	fw = 1.5% dw = 2.2%	fw = 1.8% dw = 8.3%	fw = 2.6% dw = 10.8%

Owing to the large moisture contents in "die-back" sites, values are expressed both on a dry weight and a fresh weight basis, to obtain a fair comparison.

β) Discussion.

Only the nitrogen levels varied on a fresh weight basis between the healthy sward soils (0.003%) and "die-back" soils (0.10 - 0.13%). This variation was in opposite sense to the variations in plant nitrogen levels, and hence showed that these were not important. The likelihood of mineral deficiency as a cause of "die-back" in Spartina was thus considerably reduced. Elements other than nitrogen showed a remarkable uniformity in their levels in the soil on a fresh weight basis. This is almost certainly due to the influence of the tides, maintaining a practically constant level of minerals in the soil.

(b) Minor elements.

(i) Plants.

α) Results.

The results of the minor element determinations carried out by Mr. LeRiche of Rothamsted are summarised in Table IX.

β) Discussion.

There were no marked minor element deficiencies or accumulations, but some of the results below are of interest. Elements apparently fall into four groups. Copper and manganese are both present in all plants in considerable quantities, and this may well be explained by the fact that these elements are essential to the plant, and there is some specific mechanism for their uptake. Both elements are present in slightly greater quantity in the healthy plant than in the "die-back" plants.

TABLE IX.

Mineral contents; parts per million on dry weight basis.

Site & Plant parts	Element									
	Cu	Mn	Mo	Co	Ni	Cr	V	Pb	Sn	Zn
Healthy shoots & leaves	13	40	0.3	0.5	5	5	3	5	1	35
Healthy roots & rhizomes	20	50	4	2	9	13	20	20	2	90
Channel "die-back" shoots & leaves	13	25	0.6	0.6	7	5	6	5	2	40
Channel "die-back" roots & rhizomes	20	40	6	1	6	2	14	5	1	30
Pan "die-back" shoots & leaves	6	25	0.4	0.7	4	4	4	1.5	1	25
Pan "die-back" roots & rhizomes	14	40	3	3	4	3	7	1.5	1	15

The second group of elements is that including molybdenum, cobalt, nickel and tin. These elements, especially tin, are present in such small quantities that not only are they most unlikely to be important to the plant, but there is probably no definite means for their uptake.

The third group includes chromium, lead and zinc, and these elements are present in greater quantities in the healthy plants than in the "die-back" plants. Since nothing is known of the metabolism of these elements in plants, it seems possible that their different concentrations merely reflect some other factor in operation - possibly the physical soil condition of the healthy site differs from that of the "die-back" sites in such a way as to make these elements more readily available.

Fourthly, the element vanadium is seen to be present in quite large quantity, approximately forty thousand times that present in sea water. A similar case is known in the Ascidia, which were investigated by Webb (1939), even higher concentrations (two to forty times that in Spartina) being detected. Vanadium has recently been shown to have a specific role in plant metabolism (Pirson, A., 1955).

It is concluded that apart from the minor variations in chromium, lead and zinc, none of the minor elements appears to vary in a way suggesting that a mineral deficiency is occurring. Those elements are regarded as unimportant, so that, on this evidence, mineral nutrition is considered to play no part in "die-back" of Spartina.

(ii) Soils.

α) Results.

Simple quantitative tests showed that both iron and manganese were present in the soils in all sites.

β) Discussion.

Soils normally contain ample quantities of the minor plant nutrients, and this must be especially true of soils which receive regular tidal inundations, but Webb (1939) showed that though vanadium was present in quantity in marine clay, it was not in an available state. This may be the case with other elements. Their presence in the plants in all sites though, suggests that most elements are readily available.

The two elements which may be leached out of wet soils most easily, however, and with which unbalance might be concerned, are iron and manganese (Pearsall, W.H., 1950). Both of these elements were found in all the soils examined.

D. <u>Conclusions.</u>

Neither the fertilizer trials nor the plant and soil analyses produced any evidence in support of mineral unbalance being the cause of "die-back". Application of fertilizer was without effect on "die-back", though it apparently resulted in a slight increase in "die-back" plant weight.

Mineral analyses of the plants and soils showed no consistent unbalances. Nitrogen, though low in "die-back" plants, was high in "die-back" soils. Potassium, though high in the active above ground parts of healthy plants, was little different in total concentration in the plants of healthy and "die-back" sites. It was evident that these minerals were only reflecting the unhealthy state of "die-back" plants.

Though the minor elements showed variations in concentration they were apparently unrelated to "die-back". The element vanadium was of interest in being present in some quantity.

It was concluded that mineral nutrients in the soils were not concerned in "die-back", and accordingly attention was focused on other factors in the soil which might be causing "die-back".

VI. INVESTIGATION OF THE SOIL.

A. Introduction.

It seemed possible that there was in the soil of "die-back" sites some factor harmful to Spartina growth, and an investigation into this possibility was begun. On ecological grounds it seemed unlikely that widespread pollution was causing "die-back", but it was still possible that toxic substances were present in the soil. "Die-back" of Bromus inermis was shown by Benedict (1941) to be due to toxic substances in the old Bromus roots, and there have been other well authenticated examples of the existence of toxic exudates from plants (Bonner, J., 1950). Alternatively, toxic substances might be produced during decomposition by soil bacteria.

In the special case of submerged soils new physical factors may arise which are not normally encountered in land soils. These have been suggested as giving rise to "die-back" in some few poorly authenticated examples (McDonald, M.E., 1955). New and different factors may also arise in the Spartina soils on account of the saline flooding. All these possibilities had to be taken into account in the investigation of the Spartina soil.

B. Investigation of the soil solution.

The investigation of the soil began with an examination of the properties of the soil solution, as the most likely medium for the carrying of toxins to the plant.

1. Search for substances harmful to plant life.

A germination test was applied to soil solutions to determine whether there were present in them any substances harmful to plant life generally.

(a) Materials. i) Soils.

Large soil samples (ca. 20kg.) were taken from the healthy sward site at Cocked Hat (MR 33999464), and the channel "die-back" site (MR 33409535) at Lymington.

ii) Plants.

Seeds of several plants known to withstand high salt concentration (UNESCO, 1954) were selected, together with others which germinate easily under normal conditions.
The plants chosen were:-

Wheat (Joss)	salt tolerant species.
Rye (Petkuser)	
Cabbage (Webbs King)	
Beet (Toogoods Silver)	

Maize (Early Golden)
Mustard (Toogoods White)
Cress (Toogoods Plain)

(b) Methods.

The soils were centrifuged within a few hours of collection, and the centrifugate liquid used to moisten filter paper in Petri dishes, in which the seeds were sown. The lids were replaced on the dishes, which were then placed in a glass tank beside a beaker of water, to maintain a high humidity. The lid was then sealed on the tank, which was kept at 25°C. The seeds were examined and the number which germinated was counted after one and two weeks.

(c) Results.

The results of the test are given in Table X.

TABLE X.

	Centrifugate of "die-back" soil.		Centrifugate of healthy soil.	
	Germination numbers		Germination numbers	
Seed.	One week	Two weeks	One week	Two weeks
50 Wheat	48	48	49	49
50 Rye	19	19	20	20
100 cabbage	0	19[x]	0	2
100 Beet	0	0	0	0
20 Maize	2	9	2	9
100 Mustard	0	0	0	0
100 Cress	0	0	0	0

[x] Condensation from the Petri dish lid diluted this extract after one week.

(d) Discussion.

There was, from the germination of wheat and barley, little likelihood of any substances in the soil solution being harmful to plant life. This is further supported by the slowly germinating maize. Cabbage seed is evidently intolerant of the salinity in the soils used, as dilution, in the one case where it occured, gave an anomalous result. Beet is apparently difficult to germinate, and mustard and cress are quite intolerant of salt in this concentration. The results are those that might be expected of a saline soil without toxins, the "die-back" soil being no different from the healthy sward soil.

2. Search for substances harmful to Spartina.

A similar germination test to that above was carried out using Spartina "seed" to detect any substances in the soil solution which might be specifically harmful to Spartina.

(a) Materials. i) Soils.

Soils were taken from the same healthy and "die-back" sites as in the previous experiment, and the soil solution extracted by centrifuging.

ii) Plants.

Spartina seed material which had been stored cold over winter, and which might be expected to germinate uniformly, was used in this experiment.

(b) Methods.

The centrifugate liquids were used to moisten filter paper in Petri dishes on which the seeds were sown. Two additional controls were set up, one with sea water, and the other with distilled water, to check the effects of salinity on germination.

(c) Results.

The results of the test are given in Table XI.

TABLE XI.

Germination of twenty _Spartina_ seeds, in each test solution.

Germination	Centrifugate of healthy soil.	Centrifugate of "die-back" soil	Sea water control	Distilled water control.
after 2 weeks	3	3	5	7
after 4 weeks	4	4	7	8

(d) Conclusions.

There was no difference between the germination of _Spartina_ seeds in the centrifugates of "die-back" soil and healthy sward soil, so that it was concluded that there was no substance harmful to the growth of _Spartina_ present in the "die-back" soil solution, and not present in the healthy sward soil solution.

The higher germination in the sea water control was unexpected and implied that some dilution must have occurred, or that there was some slightly retarding substance present alike in the healthy sward and the "die-back" soil solutions. The highest germination occurring in the distilled water control was in accordance with earlier findings that salinity retarded germination.

C. Investigation of the whole soil.

While there was no toxic substance in the soil solution extracted by centrifuging, it seemed possible that there might be other factors in the "die-back" soils toxic to plants, yet not extracted by centrifuging. Factors governed by the physical conditions of the soils might be of this kind. Similar tests to those carried out on the soil solution were employed on the whole soil, in the laboratory.

1. Search for substances harmful to plant life.

(a) Materials. i) Soils.

Soils were collected from the same healthy sward site and channel "die-back" site as in the previous experiments.

ii) Plants.

Joss wheat seeds were used as the test plants in this experiment, owing to their evident salt tolerance, as shown in the previous experiments.

(b) Methods.

The seeds were set in the test soils in six inch pots standing in saucers, in a sealed glass tank, containing a beaker of water to maintain a high humidity. The tank was kept at 25°C.

(c) Results.

After a month, of 25 Joss seeds set in each condition, 10 germinated in the "die-back" soil, and only 7 germinated in the healthy soil.

(d) Discussion.

Under these conditions there was clearly no substance causing harm to the plants in the "die-back" soil. The soil had, however, dried out considerably, despite the beaker of water in the tank beside the pots. The drying out greatly altered the physical condition of the soils by the end of the experiment. The slightly higher germination in the "die-back" soil was probably a consequence of the greater humidity of that soil.

2. Further search for substances harmful to plant life.

(a) Materials and Methods.

The materials used in this experiment were exactly similar to those used in the previous experiment. The method differed in that instead of sealing the pots in a glass tank, they were stood in polythene bags, which were closed at the top with elastic bands. The bags were just big enough to hold the pots. To compensate for the gravity draining of the pots, glass distilled water was supplied to them at the rate of 10 ccs. daily.

(b) Results.

All the wheat seed died, in both the healthy and the "die-back" soils.

(c) Discussion.

The soil humidity in this experiment much more closely resembled that in the field. The physical structure was little altered between the beginning and the end of the experiment, which was thus more reliable. At the same time, the experiment demonstrated that tests with other plants could not be considered completely reliable, owing to the conditions of salinity, and other factors present in the Spartina soils, making them inhospitable to other plants.

3. Search for substances harmful to Spartina.

In the search for substances harmful to Spartina, seed material was first used, in a similar manner to that used on wheat seed in the previous experiment, when the field conditions were most nearly reproduced.

(a) Materials. i) Soils.

Soils were taken from the healthy sward, the channel "die-back", and pan "die-back" sites, at Lymington.

ii) Plants.

Spartina seed material which had been stored over winter and was thus likely to give a uniform germination, was used in this test.

(b) Methods.

The method used in the previous experiment, of standing the pots in polythene bags, and supplying 10 ccs. of glass distilled water daily, was repeated in this experiment.

(c) Results.

From 20 Spartina seeds used in each test, the results were obtained which are seen in Table XII.

TABLE XII.

	Healthy sward.	Channel "die-back"	Pan "die-back"
Germination.	6	11	6
Growth	Slow and weak	Fast and strong	Fast and strong
Survival	20 weeks	16 weeks	25 weeks.

(d) Discussion.

The results show that germination, growth and survival were generally better in the "die-back" soils than in the healthy sward soil (Fig. 25). This is assumed to be an effect of the greater soil humidity in the "die-back" soils.

FIG. 25. Growth of *Spartina* seedlings in soils from healthy and "die-back" sites. Note strong growth in "die-back" soils.

The fact that in no soil did the seedlings survive is not regarded as significant owing to the difficulties previously experienced in the growth of Spartina other than in sand culture, with fresh water supplied. The failure may safely be attributed to the effects of salinity and change of soil structure during the experiment. No way of overcoming these difficulties was discovered.

4. Further search for substances harmful to Spartina.

The test on seedlings left some doubt as to the effects of the soil on the Spartina plant. This test had proved unsuccessful both because of the eventual death of all the seedlings, and also since the seedlings had evidently been responding to the humidity of the soil rather than the chemical conditions in the soil. It was doubted whether the seedlings, with their shallow roots, and with no rhizomes, were giving a true reflection of the soil conditions below the immediate surface layer. To test the effects of the deeper layers of soil on the Spartina plant, offsets were planted instead of seedlings, and their response to the soil conditions noted.

(a) Materials. i) Soils.

Soils were taken from the healthy sward site, and from the channel and pan "die-back" sites, at Lymington.

ii) Plants.

Spartina offsets from the healthy sward site were taken, each as nearly as possible the same size as the others, and brought back to the laboratory.

(b) Methods.

The offsets were planted in six inch pots of the test soils in polythene bags, as in the previous experiments. The bags were then closed around the shoots with elastic bands. Glass distilled water was supplied at the rate of 25 ccs. daily.

(c) Results.

The results are seen in Table XIII.

TABLE XIII.
Growth of Spartina plants in soils.

	Healthy sward	Channel "die-back"	Pan "die-back"
Growth.	Fast and strong	Slow and weak	Slow and weak
Survival	Strong at 25 wks.	Weak at 25 wks.	Dead at 8 wks.

(d) Discussion.

The results of growth of offsets resembled "die-back", giving the first indication of the "die-back" mechanism, and showing that it was almost certainly a soil effect (Fig. 26), associated with the soil solids or physical soil structure.

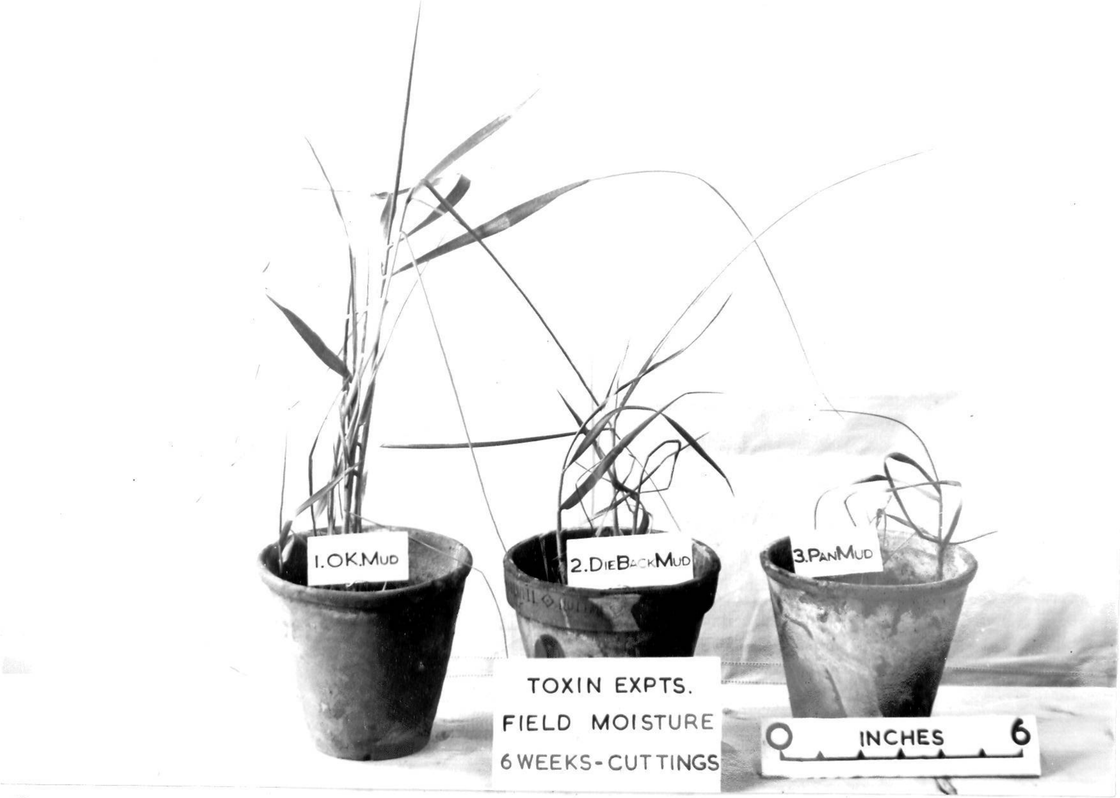


FIG. 26. Growth of *Spartina* plants in soils from healthy and "die-back" sites. Note strong growth in healthy soil.

5. Discussion of the results of the search for toxins.

There was apparently some toxic factor associated with the whole soil, yet not found in the soil solution, suggesting that the soil solids were involved, or that the toxic effect was brought about by the physical structure of the soil. This was further supported by the fact that seedlings, whose growth scarcely penetrated below the surface of the soil, were not affected by the factor.

The absence of any detectable toxin in the soil solution and the lack of any effect on seedlings of *Spartina* suggested that no toxic exudate of the kind found in *Bromus inermis* by Benedict (1941) was present.

Isolation of the factor involved was made difficult in growth experiments by the special conditions of the soil, by its salinity, and especially by its flocculated clay structure, which was most noticeable in the "die-back" soils. The structure of the soil was difficult to preserve under laboratory conditions.

The fact of the peculiar nature of the soil structure and its variation between healthy and "die-back" sites, being less humid and coarser in the former than in the latter, suggested that an investigation of the physical soil conditions might prove profitable.

D. Physical structure of the soil.

Investigation of the physical nature of the *Spartina* substrate was suggested by Austwick (1950) and was further rendered necessary by the findings of the search for toxins. Accordingly, an investigation was begun into the soil moisture content, organic matter content, and mechanical structure, in relation to "die-back".

1. Materials.

Samples of the top 5 cm. of soil were taken from each of the sites chosen in the previous investigations, namely, the healthy sward site (MR 33999464), the channel "die-back" site (MR 33409535), and the pan "die-back" site (MR 33409540).

2. Methods.

(a) Moisture determination.

The samples were stirred, and moisture contents determined by oven drying at 90°C. After satisfactory moisture determinations had been made, the whole samples were dried, ground in a mortar, and stored in screw-topped jars.

(b) Loss on ignition.

Loss on ignition was determined by heating specimens of the dry soil to 950°C. in a muffle furnace, cooling in a desiccator, and repeating, until no further loss in weight occurred. The method was used for measurement of organic matter, as oxidation methods proved inaccurate and generally unsatisfactory.

(c) Mechanical analysis.

The standard "beaker" method of analysis described by Piper (1950) was used for the first attempts at mechanical analysis of Spartina soils. Satisfactory destruction of organic matter was achieved by treatment with hydrogen peroxide, but all attempts at dispersion failed. The soil remained flocculated throughout the treatments with electrolytes. Sedimentation methods of analysis were finally discarded in favour of sieving methods, similar to those used by Jacquet (1949). 10 g. samples of dried soil were washed through the sieves, Nos. 40, 60, 80, 100, 140, 180, 200, 250 and 300, which were accurately calibrated. Washing was performed with sea water. A final fraction was collected on filter paper. A small amount of colloidal material passed through the filter papers, but its weight was negligible. Use of sea water for washing prevented flocculation which occurred in fresh water. The results were not of the highest degree of accuracy, but the method has more recently been refined by Miss E.M. Uren (unpublished).

3. Results.

(a) Moisture contents.

The results, from quadruplicate samples taken both as the tide left the <u>Spartina</u> sward, and after it had been low for some time, to give an indication of the maximum variation, are given in Table XIV.

TABLE XIV.

	Healthy sward		Channel "die-back"		Pan "die-back"	
	After high tide	At low tide	After high tide	At low tide	After high tide	At low tide
Moisture content (%)	25.7	30.7	84.0	77.8	80.1	75.8
Dry weight (%)	74.3	69.3	16.0	22.2	19.9	24.2

Determinations in other areas where "die-back" was occurring showed that the lower water content of healthy sward than "die-back" sites was a completely general phenomenon.

(b) Losses on ignition.

The results of the loss on ignition determination will be seen in Table XV.

TABLE XV.

	Healthy sward	Channel "die-back"	Pan "die-back"
Loss on ignition	5%	30%	24%

As with the water contents, analyses of other sites showed that the differences between "die-back" soils and healthy sward soil in ignition loss were quite general.

(c) Mechanical analysis.

The results of the mechanical analyses are shown diagrammatically in Fig. 27, and to these results, for completeness, have been added those of loss on ignition. Mechanical analyses in other areas have shown that the type of results here are quite general for "die-back" areas.

4. Discussion.

There are striking differences in soil structure between the healthy sward on the one hand and the "die-back" swards on the other. Healthy sward soil had a larger silt fraction and a very much smaller water content, ignition loss, and colloidal fraction, than the "die-back" soils. Equally noticeable is the great similarity between the two "die-back" soils. The soil structures are evidently of primary importance, since they differ in the same sense as growth in the soil toxin tests, and as "die-back" itself.

There can be little doubt that the differences in soil water content are a result of the differences in the soil colloid and organic content. The large differences in water content, however, are comparable rather with the large differences in organic matter (loss on ignition) than

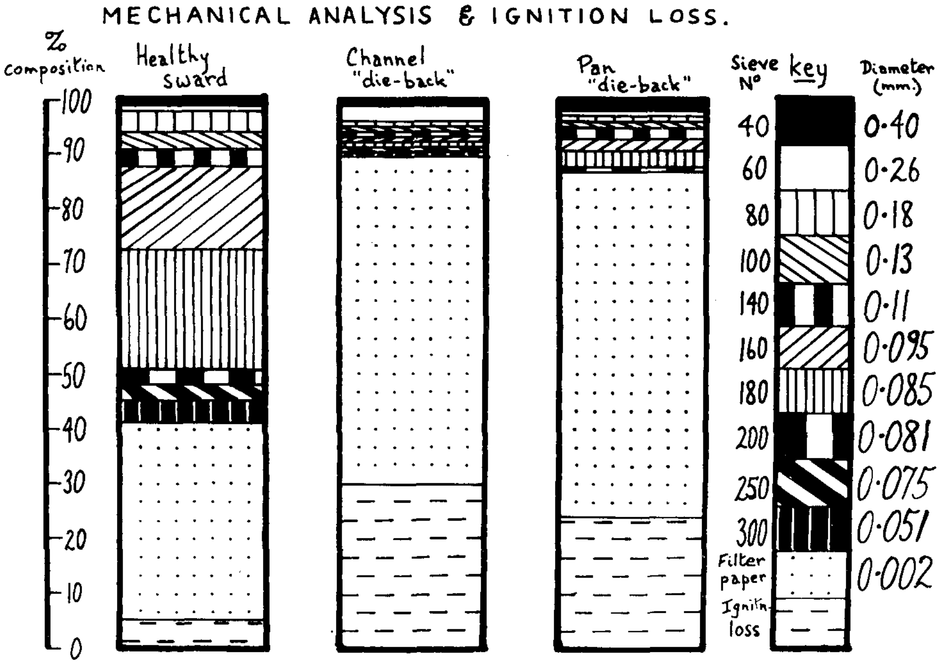


FIG. 27. Mechanical analyses of Spartina soils, with losses on ignition. Note the strikingly different losses on ignition and colloidal fractions in healthy and "die-back" soils.

]

with the smaller differences in the colloidal fraction.

The organic matter of the soil arises from Spartina decay, and cannot therefore be a primary factor in water-logging, and the "die-back" it may cause. It seems that colloid accretion may cause a rise in water content in the first place, and if this initiated Spartina decay, the organic matter in the soil would increase, and hence the water content, more Spartina would die, and the process would be self regenerating.

Such a system would develop gradually at first, from the arrival of Spartina in a suitable area. Once "die-back" began, however, it would become faster and faster, and the observed result would be sudden and catastrophic. Certain chemical reactions are characterized by an initial apparently quiescent period of induction, followed by a sudden change, and these have been shown to involve chain mechanisms or autocatalysis, both, in a sense, consecutive reactions. A typical example is seen in the autocatalytic reaction of potassium permanganate, sulphuric acid, and oxalic acid. The reaction at first proceeds slowly, but soon becomes very rapid, as manganous ions accumulate from the reaction, and act as a catalyst for it (Glasstone, S., 1951). It is considered possible that "die-back" may proceed in this way.

E. Conclusions.

A factor harmful to Spartina growth and associated with the whole soil was revealed by toxin investigation. Examination of the physical structure of the soils showed that they differed greatly in the same sense as in the toxin experiment growth failure, and as in "die-back". The effect of the differences in soil structure was a great increase in the water content of the "die-back" soils. This was shown to be self regenerating.

For waterlogging to cause "die-back", however, it must be associated with the removal of some substance essential to the plant (for example oxygen), or the accumulation of some toxic substance. In certain circumstances bacterial activity gives rise to toxic substances in waterlogged soils (Pearsall, W.H., 1950). To determine whether this was the case it was essential to carry out a full investigation of waterlogging, soil aeration, and the products of bacterial activity.

VII. THE EFFECT OF WATERLOGGING ON PLANTS.

A. Introduction.

The suggestion that waterlogging was associated with *Spartina* "die-back" made necessary a study of the reaction of the plant to waterlogged conditions. The regular tidal inundations received by the plant in the habitat made artificial waterlogging experiments possible. It was from field observations that deficient soil aeration was postulated as a cause of root rot in rice (van Raalte, M.H., 1940).

A preliminary study of *Spartina* growth in relation to waterlogging was made in the field, prior to detailed work in the laboratory.

B. Field trials - Draining and waterlogging.

1. Methods.

Three incipient pan sites were studied in the area MR 340950 at Lymington. One, the control, was that chosen in the study of sward morphology in relation to panning (Section IV above). The other two incipient pan areas were chosen for their nearness to drainage channels. All were sampled in September 1955 before the experiments began, above and below ground growth being measured.

The first of the experimentally treated incipient pans was a low area kept in a waterlogged state by a complete levée bank separating it from a nearby channel. In this experiment a gully was dug through the levée, allowing the tide to run off at each ebb. Sward sampling was carried out at two monthly intervals for a year, until August 1956.

The second experimentally treated incipient pan was a similar low area of the sward inside a levée bank, but in this case the bank had been broken in several places by the tide. The *Spartina* growth in the area was poor, and hence it seemed likely that the breaks were of recent origin. These breaks were repaired by laying *Spartina* turf across them. In a short time the turves took root and the area lay under several inches of water throughout each intertidal period. Sward sampling was carried out at two monthly intervals for a year, until August 1956. Details of the control incipient pan have been given in Section IV.

2. Results.

Over the period of observation (September 1955 to August 1956) the behaviour of neither the drained nor the waterlogged incipient pan differed markedly from the control. In all three there were the same seasonal trends - short, few leaved rosettes in the spring, and tall, many leaved culms in the summer. There were no marked differences in shoot numbers, though there may have been, in August 1956, a slight improvement in the above ground growth of the drained site and a slight deterioration of the waterlogged site. The effects were small, and were not reflected in the behaviour of the below ground parts. This, and the fact that they were not detected until the end of the period of observation makes their value doubtful. Detailed results are given in Appendix 3.

3. Discussion.

It is evident that incipient pan drainage does not effect an immediate improvement in Spartina growth, nor does incipient pan waterlogging effect an immediate decline.

Although on the basis of the field trials there was little evidence for the association of waterlogging and "die-back", on ecological grounds there seemed to be strong evidence, so that reasons may be sought for the failure of the field trials to demonstrate this. The field trials may have been inconclusive on account of a time lag, either in the setting up of the habitat conditions, or in the response of the plants to them. Waterlogging is most unlikely, in itself, to cause "die-back". Rather, the consequences, such as anerobiosis and bacterial activity, are likely to cause it. These might not immediately follow flooding.

It might be, too, that bud failure would not occur immediately at the onset of adverse conditions. The most severe results of bud failure might well be delayed until the flowering stems of the following year had died down. If these flowering stems were not replaced, the failure might not be detected until February 1957.

The field observations on rice were also inconclusive, and it was for this reason that attention was directed to laboratory studies on that plant, in relation to anaerobiosis, by van Raalte (1940, 1943). With this previous work in view, the study of Spartina anaerobiosis was begun in the laboratory.

C. Examination of the aeration system of Spartina.

1. Introduction.

Oxygen is required by the roots and rhizomes of plants, or their growth is affected. Hydrophytes seldom suffer from lack of oxygen, and this is associated with aerenchyma, which, it is supposed, allows gas diffusion inside the plants (Bergmann, H.F., 1920). Conway (1937) first studied the continuity of such systems, and their resistance to gas flow. The same author, by analysing the gas contents in successive spaces along the plant organs, demonstrated the ease of gas diffusion in such systems.

Recent studies have shown that some plants can remove CO_2 from their rhizomes by fixing it as lactic acid, and a high level of anaerobiosis can be maintained (Barber, D., unpublished). The investigation of Spartina anaerobiosis set out to study the continuity of the aeration mechanism of the plant, the ease of gas diffusion, and the type of metabolism, by the available techniques. Particular attention was paid to the rhizomes, since the chief character of "die-back" was the rhizome apex failure.

2. Resistance of the aeration system.

(a) Simple porometer.

The simple porometer was first used to measure the ease of gas flow through the rhizomes of the Spartina plant. Healthy rhizomes from the Cocked Hat area at Lymington were used.

(i) Methods. α) Rhizomes.

The rhizomes were fitted loosely into lengths of glass tubing, slightly shorter than the rhizomes to allow for sealing with luting wax. The space between the tubing and the rhizomes was filled with mercury to prevent leaks. Mounting in this way allowed rhizomes to be attached to the porometer by rubber tubing.

β) Porometer.

By means of an aspirator, air was drawn through a moistened cotton wool pad of negligible resistance, through the rhizome, and over a manometer. The manometer measured the pressure on the aspirator side of the rhizome, the other end being at atmospheric pressure. A length of wire ran through the cotton wool pad on the atmospheric pressure side, and this was used to push through the successive diaphragms of the rhizome.

ii) Results.

In every experiment with the simple porometer the result was the same. Rhizomes with up to six diaphragms had these punched out successively. Each time the rise in the pressure measured by the manometer on punching the diaphragms was negligible until the last diaphragm was reached, when the pressure became atmospheric throughout the apparatus.

iii) Discussion.

The result is that to be expected if each diaphragm had a very high resistance. The current of air flowing through the apparatus was so small, the resistance of each diaphragm being nearly infinite, that the pressure changes were negligible, as successive diaphragms were punched. When the last diaphragm was opened, however, there was a rapid rise to atmospheric pressure, resistance having been removed almost entirely. To measure the very high resistances of the diaphragms a resistance porometer had to be used.

(b) Resistance porometer.

i) Methods.

Rhizomes mounted in the same way as before were used in the resistance porometer modified from that described by Gregory and Pearse (1934) and used by Professor W.T. Williams and kindly lent by him for this purpose.

ii) Results and Discussion.

The resistance porometer confirmed the high order of the resistance of single rhizome diaphragms of Spartina. Each diaphragm had a resistance comparable to that of a one centimetre length of thermometer tubing. Such a resistance must greatly hinder diffusion. The reasons for the high resistance, and the results of it, had to be further examined.

3. Structure and development of the aeration system.

A solid cone of nodes about one millimetre long lies behind the vegetative apex of Spartina, bearing the sheathing leaves. Behind the cone, medullation begins. In the centre of the rhizome the nodal tissue gives way to loosely packed parenchyma cells which break down to form the medullary air space of the first elongated internode. Around the medullary air space, about two millimetres behind the apex, cortical air spaces form. There are usually 15 - 20 of these. The first elongated internode is usually 2 - 3 mm. long.

At the nodes the cortical and medullary spaces are filled by parenchyma with small air pores, the cells having the shape of an irregular several armed figure, similar to those seen in Zea Mays by McPherson (1939). These cells, supported by vascular strands, form a complete diaphragm at each node. The diaphragms are more complete than in most aquatic plants, and this accounts for the high resistance of the Spartina rhizome to air flow (Fig. 28).

Medullation was considered by McPherson (1939) to be promoted by anaerobic respiration. The relation between anaerobiosis and medullation is of interest in Spartina, and is the subject of a study by Miss F. Hopgood (unpublished). Meanwhile, investigation was continued on the more general aspects of anaerobiosis.

4. Gas composition in the aeration system.

(a) Methods.

Rhizomes were taken from Spartina plants in the field, pinched at the ends and sealed with luting wax to prevent entry of air. They were kept under water for the shortest possible time before being transferred to the gas extractor.

Samples of the gas were withdrawn from the air space system by a vacuum extraction apparatus which was a modification of that used by Vallance and Coult (1951). The analysis of the internal atmospheres for oxygen, carbon

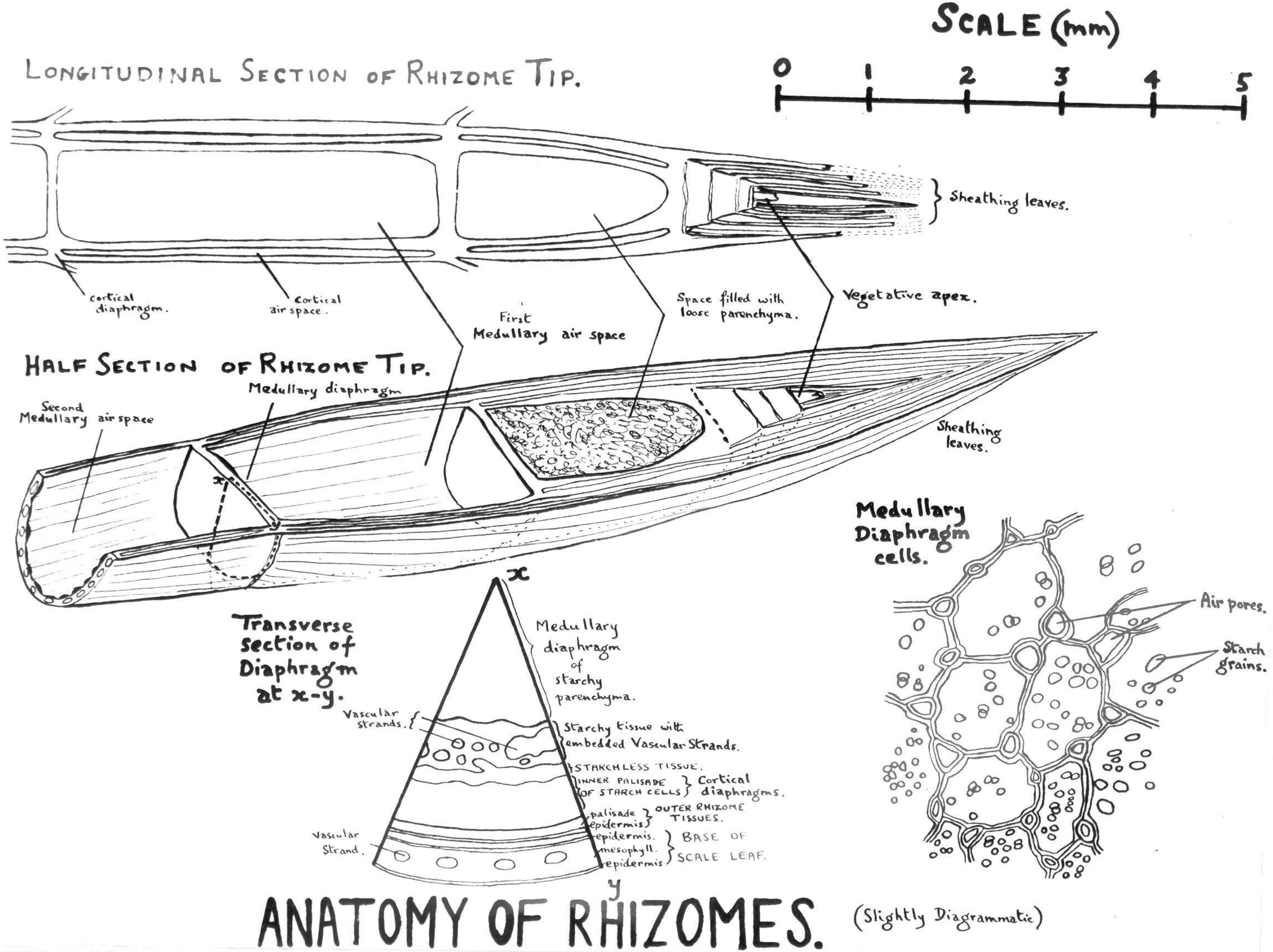


FIG. 28. Structure of the Spartina rhizome. Note the diaphragms, with only very small air pores between the cells.

dioxide, and nitrogen was carried out in a similar apparatus to that used by Vallance and Coult (1951). Duplicate samples of approximately 100µl. were analysed to give each result. The sensitivity of the apparatus is such that carbon dioxide determinations on air give results not exceeding 0.5%, (theoretical value 0.0% within the limits of the apparatus), while there is no significant difference between the oxygen concentrations determined by the Haldane and Vallance methods (Vallance, K.B., & Coult, D.A., 1951).

All analyses were completed within six hours of sampling, to avoid possible errors due to interference by water and oxidizable impurities in the mercury, which cannot be completely eliminated in this method.

(b) Results.

i) Laboratory experiments.

Preliminary experiments carried out under the guidance of Mr. D.A. Coult showed that the Spartina plant had more oxygen and less carbon dioxide at the proximal ends of the rhizomes than at the distal ends. The observation could not be repeated owing to the small amounts of gas which could be extracted from single rhizomes. Also in the preliminary experiments it was shown that plants from the Dee estuary with an initial concentration of 6% CO_2 and 15% O_2 produced 12% CO_2 and 2% O_2 after 24 hours under oil - a very rapid response to the anaerobic conditions.

Experiments on plants kept in laboratory sand cultures showed that the levels of oxygen and carbon dioxide were similar to those found in the sandy Dee estuary (3.9 - 4.8% CO_2 : 11.8 - 14.1% O_2).

ii) Field collections.

Plants were collected from the healthy sward area at Cocked Hat, Lymington (MR 340946), the area of channel "die-back" near the Pier (MR 334954), and the area of pan "die-back" near the Pier (MR 334954), and analyses of their internal atmospheres were carried out. The results are shown in Table XVI.

The variations in the number of observations in the tables are due to the ready availability of the necessary long rhizomes with large enough gas contents for duplicate analyses in the channel and pan "die-back" sites, and their scarcity in the healthy site, where the rhizomes were generall shorter and quickly upturning. The fact that only long rhizomes gave sufficient gas for analysis - and these were the less healthy ones - tended to reduce the differences between the healthy and the "die-back" sites.

(c) Discussion.

The same positive trend as was noticed in the toxin tests and soil structures was seen in the average oxygen contents of the rhizomes. Healthy sward rhizomes had a

TABLE XVI.

Percentage CO_2 and O_2 in the internal atmospheres of rhizomes.

		Healthy sward			Channel "die-back"			Pan "die-back"		
7-2-56	O_2	0.0	-	-	0.3	2.3	0.7	0.5[x]	3.2	2.9
	CO_2	0.9	-	-	4.1	5.4	4.1	8.0	3.9	2.3
25-2-56	O_2	0.0	2.3	2.7	3.9	3.1	3.2	0.0[x]	1.1	0.0[o]
	CO_2	5.2	3.6	5.4	2.6	1.4	1.6	9.5	1.6	5.8
5-3-56	O_2	-	-	-	-	-	-	3.0	1.9[x]	-
	CO_2	-	-	-	-	-	-	8.2	7.1	-
27-6-56	O_2	5.9	6.6	1.8[x]	0.0[o]	1.9	-	0.0[x]	0.3[x]	-
	CO_2	8.1	6.8	10.7	5.8	4.5	-	6.8	10.5	-
		avg. O_2=2.8: CO_2=5.8			avg. O_2=1.9: CO_2=3.7			avg. O_2=1.3: CO_2=6.4[x]		

[x] Values exceeding toxic level for *Menyanthes* (Coult, D.A. unpublished)

[o] Values approaching toxic level for *Menyanthes*.

higher average oxygen content than those of "die-back" swards. The lowest average content was found in pan "die-back" rhizomes. Differences in the carbon dioxide content were not as markedly positive, channel "die-back" rhizomes containing on average less CO_2 than healthy sward rhizomes.

Carbon dioxide concentrations of over 6% in combination with oxygen concentrations below 2% are toxic in Menyanthes (Coult, D.A., unpublished), the plants dying on account of a CO_2 stimulation effect on respiration. Laing (1940), examining many aquatics, found several which had high rhizome CO_2 contents in the field, but oxygen contents were never below 2% unless the plants were artificially shaded. No toxicity values are known for Spartina, but values exceeding the toxic level for Menyanthes have been marked (x), and those nearly approaching it (o), in Table XVI. These marks clearly show the probably toxic levels of CO_2 in the pan "die-back" site. The channel "die-back" and healthy sites are less different, but the former, with an average O_2 concentration below 2% may more nearly approach a generally toxic level than the latter. The high average oxygen level in the healthy sward site makes any general toxicity unlikely.

Far lower rhizome oxygen contents are the rule at Lymington than in the Dee, or in sand culture. This strongly suggests that conditions of aeration at Lymington fall far short of the optimum for Spartina growth. Anaerobic respiration is obviously an occasional feature of the healthy sward rhizomes, a more common occurrence in the channel "die-back" rhizomes, and the predominant feature of the pan "die-back" rhizomes. Further investigation was carried out on the products of anaerobiosis.

5. Products of anerobiosis.

An exploratory investigation was begun into the products of anaerobic respiration in the Spartina rhizomes. That these were present at least in the "die-back" rhizomes was suggested by an ester-like odour not noticed in the healthy rhizomes. The major products of anaerobiosis are normally alcohol and acetaldehyde (Gustafson, F.G., 1934), and measurements were made of these.

(a) Methods i) Distillation.

The reduced pressure distillation method of Gustafson (1934) was used to collect the alcohol fraction from samples of 10 - 30g. of rhizomes (7 - 16, as available). The alcohol fraction was collected in sulphuric acid.

ii) Acetaldehyde.

An aliquot of the distillate was neutralized with sodium carbonate, and tested with Fehling's solution for the presence of aldehydes.

iii) Alcohol.

Further aliquots of the distillate were added to standard potassium dichromate solution, excess potassium iodate was added, and the iodine liberated was titrated with standard sodium thiosulphate solution.

(b) Results. i) Acetaldehyde.

No positive reactions were obtained from the distillates when treated with Fehling's solution.

ii) Alcohol.

The results of the alcohol determinations are given in Table XVII.

TABLE XVII.

Percentage alcohol (fresh weight basis).

	Healthy sward rhizomes.	Channel "die-back" rhizomes.	Pan "die-back" rhizomes.
21-12-55	-	2.8×10^{-2}	4.6×10^{-2}
3-1-56	4.6×10^{-3}	1.5×10^{-2}	1.15×10^{-1}
29-6-56	5.4×10^{-2}	1.36×10^{-1}	1.99×10^{-1} 1.26×10^{-1}
	avg. 2.9×10^{-2}	avg. 6.0×10^{-2}	avg. 1.22×10^{-1}

(c) Discussion.

There is a strong correlation inversely between the alcohol levels in the rhizomes and their oxygen contents. <u>Spartina</u> can apparently fix no CO_2 as lactic acid, alcohol being the product of anaerobiosis. The results obtained entirely confirm the trend of anaerobiosis at Lymington, being rare in the healthy sward, more marked in the channel "die-back" sward, and predominant in the pan "die-back" sward rhizomes.

D. Conclusions.

Although experimental draining and waterlogging did not immediately affect the growth of *Spartina*, laboratory investigation showed that the aeration system of *Spartina* is not as efficient as is usual in aquatic plants. The diaphragms between air spaces are very resistant to passage of air. Although high rhizome oxygen contents are found in the *Spartina* of the Dee, and in sand culture, low rhizome oxygen contents are the rule at Lymington. Healthy sward rhizomes respire mainly aerobically, channel "die-back" rhizomes less so, and pan "die-back" rhizomes are predominantly anaerobic. These differences must arise from variations in the reducing power of the habitat, and this was next examined.

VIII. THE EFFECT OF WATERLOGGING ON SOILS.

A. Introduction.

Variations in the reducing power of the soils were suspected from the observations on the anaerobiosis of plants in response to waterlogging. Support for this conclusion was gained by examination of the soils. Healthy sward soil was greyish, with brown stains, showing that both ferrous and ferric iron were present, while "die-back" sward soils were black from ferrous iron, with no brown stains, and smelt strongly of sulphides. Sulphur bacteria were abundant in the black soil (Clitheroe, A., 1955).

The state of reduction of soils may be measured either by physical or by chemical methods. The theoretical considerations of the important physical technique of measurement of redox potential were discussed by Pearsall (1938), and the relation of the potentials to the aeration of the soil noted. Chemical determinations have mainly employed redox indicators, though occasional direct dissolved gas determinations have been made (Scholander, P.F., van Dam, L., & Scholander, S.I., 1955). Both redox potential measurement and direct dissolved gas determinations were attempted on the *Spartina* soils.

B. Oxidation-reduction potentials.

1. Method.

The type of platinum strip electrode used by Mortimer (1941) was employed against a calomel half cell and the potential was measured with a pH meter manufactured by the Cambridge Instrument Company. Measurements were also made of the pH with a glass electrode.

Both measurements were made in situ at low tide, after the electrodes had been in the mud for around 20 minutes. The mud was scarcely disturbed by the treatment, so that the values obtained should reflect the true state of the soil.

2. Results.

The results of the measurements of redox potential and pH are shown in Table XVIII.

TABLE XVIII.

	Healthy sward		Channel "die-back"		Pan "die-back"	
	Redox	pH	Redox	pH	Redox	pH
Surface	250mV.	6.8	xHighly -ve	6.6	xHighly -ve	6.6
10 cms.	180mV.	6.8	xHighly -ve	6.6	xHighly -ve	6.6

xNo stable values obtainable.

3. Discussion.

There is a very marked difference between the order of the redox potentials in the healthy and "die-back" sites, in the same direction as the differences already observed in the toxin tests, the soil structure, and the plant anaerobiosis. Mortimer (1942) showed that progressive reduction in muds from ferric to ferrous iron occurred at an oxygen concentration of 0.1 mg./L, and sulphate to sulphide at an oxygen concentration of zero. The oxidation - reduction potentials accompanying these changes were

$Fe^{+++} - Fe^{++}$ E_7 = 300 mV - 200 mV, and

$SO_4^{--} - S^{--}$ E_7 = 100 mV - 60 mV,

both values being corrected to pH 7. It is necessary to make corrections for the pH of the mud to compare redox potentials. No correction values have been measured for Spartina mud, but the pH difference from pH 7 is small, and errors from inaccurate correction will be minimal. Applying the correction of Pearsall and Mortimer (1939), the values of the Spartina soil redox potential at pH 7 become

E_7 (Surface) = 240mV.

E_7 (10 cms.) = 170mV.

for the healthy sward soil, the largest possible error due to inaccurate correction being - 10mV. (van Raalte, M.H., 1943). At the surface of the healthy sward, on Mortimer's values, both ferric and ferrous iron are likely to be present. This supports the previous observations. Lower down, there is likely to be only ferrous iron. "Die-back" sites are likely to contain ferrous iron and sulphides. Instability of potentials in these sites may be due to the presence of hydrogen sulphide gas.

C. Chemical determinations.

Measurements of the gases oxygen and hydrogen sulphide were made on the soils as these appeared, from the redox potentials, to be present in important quantities. Of the two, it seemed likely that hydrogen sulphide was the more important, oxygen concentrations always being very low. Redox indicators were not used, as being less informative than gas measurements, at the high levels of reduction here present.

1. Soil solutions. (a) Direct methods.

i) Methods and Materials.

A first attempt was made to measure the oxygen and hydrogen sulphide gases in the soil solution by the method of Rutter (unpublished). Doulton porous electrolytic (Diaphragm) cells (grade F 10, retaining solids down to ca. 3μ) were stoppered with rubber bungs, through which ran two glass capillary tubes - one reaching to the top of the cell, the other to the bottom. The bung and tubes were sealed in position with shellac, and a protecting tube of polythene, sealed at the lower end but bored with holes to allow water passage, was put around the cell.

The units were set near the surface, and at 10 cms.

depth, in the healthy sward, the channel "die-back" sward, and the pan "die-back" sward, and filled with distilled water which had stood in the laboratory air, attaining a pH of 4. The outlet tubes were stoppered with pressure tubing and short lengths of glass rod, and left for three weeks.

After three weeks the solutions had equilibrated with the soils, being ca. pH 7. The solutions were displaced by blowing down the shorter tube to the porous cell, and collecting the solution from the other. The specimen tube of solution was stoppered with a rubber bung, a pin being used to release all air from above the liquid.

After sampling, the distilled water in the cell was renewed, and the tubes were again stoppered.

ii) Results and discussion.

When analyses were attempted on the porous pot samples they were found to contain substances which interfered with common chemical reagents, making direct chemical determinations on the solutions difficult or impossible, and greatly impairing their accuracy. Rough tests showed that there were no more than traces of oxygen and hydrogen sulphide present in the soil solutions.

At the low levels of oxygen concentration it was doubtful whether accurate results could be obtained and, once obtained, whether they would be materially helpful to the investigation. The redox potentials, suggesting the major change to be SO_4^{--} - S^{--}, with some Fe - Fe in the healthy site, indicated that sulphide levels would more closely define the system. An alternative and more successful indirect method of gasometric analysis was designed for sulphide determinations in soils and soil solutions (Appendix 4).

(b) Indirect methods.

i) Porous cells.

α. Methods and Materials.

Solutions from the Rutter porous cells in each of the sites were used in the gas bubbler (Appendix 4), to measure the amount of sulphide they contained.

β. Results.

The solutions from the healthy sward, the channel "die-back" sward, and the pan "die-back" sward all contained traces of sulphides, of 1 - 2 x 10^{-5}g./20 ml. sample. The amounts were too small to be measured with accuracy.

ii) Centrifuging.

α. Methods and Materials.

Soil samples of about 30 g. were centrifuged, as in the toxin experiments, and sulphide was measured in the centrifugate liquid, using the gas bubbler.

β. Results.

No traces of sulphides could be detected in the centrifugate liquid.

(c) Discussion.

It was apparent that the soil solution, obtained by centrifuging, contained no detectable sulphide, while the solution in the porous pots contained only traces of sulphide. The small amount of sulphide detected in the soil solution appears to be a true value, as it seems unlikely that any quantity escaped during manipulation. There were thus apparent anomalies between the redox potentials which suggested that there was much sulphide in "die-back" sites and the actual determinations on the soil solutions which detected little sulphide. To explain these, determinations of sulphides were carried out on the whole soils.

2. Whole soils.

Duplicate determinations were made of the sulphides released by a stream of gas, and by the action of acid, in combination with a stream of gas.

(a) Materials and Methods.

Samples of the top 4 cms. of soil were collected from the healthy sward, the channel "die-back" sward, and the pan "die-back" sward. Sulphide determinations were carried out on the samples in the gas bubbler (Appendix 4). Sulphide flushed out of the soil by a nitrogen stream was first determined, then, on the same samples, after addition of hydrochloric acid, the remaining sulphides were measured. Determinations were also made using citric acid (10%) to release sulphides, in place of the hydrochloric acid. The amount released by citric acid was considered to be a measure of the sulphide available to the plant.

(b) Results.

The results of the sulphide determinations of both the soil solutions and the whole soils are shown in Table XIX.

Table XIX.

Sulphide content of soils and soil solutions.

	Healthy sward	Channel "die-back"	Pan "die-back"
Soil solution. i) Centrifuged S^{--}g./20 ml.	NIL	NIL	NIL
ii) Porous pot S^{--}g./20 ml.	TRACE (1-2x10^{-5})	TRACE (1-2x10^{-5})	TRACE (1-2x10^{-5})
Whole soil. i) N_2 stream S^{--}g./100g. soil.	NIL	6.0x10^{-5}	8.16x10^{-4}
ii) N_2+HCl S^{--}g./100g. soil	TRACE (1x10^{-5})	3.60x10^{-2}	7.54x10^{-2}

Citric acid treatment released amounts within 10% of the sulphide released by the hydrochloric acid, in different samples from the same sites.

(c) Discussion.

Sulphide contents of the whole soils provided further important positive results in support of those already obtained from the toxin tests, soil structures, and plant anaerobiosis, showing the difference between the healthy sward and the "die-back" swards. Once again, the pan area was the more anaerobic of the "die-back" sites.

An important fact emerges which may explain the anomaly of the small and apparently uniform soil solution sulphide content when there was obviously much readily available sulphide in "die-back" soils. Allowing for the measurement of solution volumes and soil weights, there was forty times as much sulphide released by nitrogen alone in the channel "die-back" soil as was detected in the soil solution, and in the case of the pan soil, the value was five hundred times as much. Mr. K.R. Butlin of the Microbiology Section of the Chemical Research Laboratory, D.S.I.R., Teddington, has pointed out that the trace quantities of sulphide detected in porous pots, $1 - 2x10^{-5}g.S^{--}/20$ ml., is reasonably close to the solubility of FeS ($2x10^{-3}g.S^{--}$/litre at 18°C.). At this low concentration of sulphide, no greater accuracy could be expected of the method used. Iron was present in quantity, and probably in excess, when practically all sulphide present would have been FeS, saturating the solution. Values recorded as nil probably represent traces which have either gone undetected at this limit of accuracy, or possibly have undergone oxidation during centrifuging.

Whereas the differences between trace quantities and nil results are not regarded as being significant, the higher values, well within the accuracy of the method, are clearly important. The healthy sward soil had no more than a trace of sulphide in any form, while the "die-back" soils had considerable quantities of sulphide blown out in the nitrogen stream, both before and after acid treatment. It is believed that the soil sulphide is in equilibrium with that in the soil solution. Blowing nitrogen through the soil solution causes the reactions:-

$$\underset{\text{dissolved}}{FeS} \rightleftharpoons Fe^{++} + S^{--}$$

$$Fe^{++} + 2OH^- \rightleftharpoons Fe(OH)_2$$

$$S^{--} + 2OH_3^- \rightleftharpoons H_2S + 2H_2O$$

to go to the right. With solution only, this proceeds until no FeS remains. When soil is present, however, the dissolved FeS is replaced from the soil "reservoir" of FeS, and considerably more H_2S is evolved. The amount of H_2S blown out by nitrogen alone is a definite and reproducible quantity for each soil. Clearly a new equilibrium is set up in the nitrogen stream - possibly controlled by the presence of excess $Fe(OH)_2$. All the remaining sulphide can then be liberated by acid.

Thus, although all soils contained traces of ferrous sulphide in solution, healthy soils had no sulphide "reservoir" capable of maintaining the dissolved sulphide level, while "die-back" soils held abundant sulphide "reservoirs" which freely supplied the soil solution. The ready availability of the "reservoir" sulphide to citric acid suggests that much sulphide may contact the plant in "die-back" conditions.

D. Conclusions.

Full confirmation was obtained of the strongly reducing nature of the soil expected from the anaerobiosis of plants in the "die-back" sward at Lymington. Healthy sward was scarcely anaerobic, having a positive redox potential in the Fe^{+++} - Fe^{++} range, and containing only the smallest traces of ferrous sulphide, both in the soil solution, and as soil solids. The reducing power of the "die-back" soil, noteworthy for its negative redox potential, was due to the presence of considerable amounts of bacterially produced ferrous sulphide in the soil solids, forming a "reservoir" which maintained the soil solution saturated with FeS. Removal of the sulphide "reservoirs" from the toxic soil by isolation of the "die-back" soil solution had already been observed to render the solutions non-toxic, (Section VI above).

Reducing conditions are associated not only with plant anaerobiosis, but with the production of new ions, including ferrous and sulphide, in the soil. Ions peculiar to waterlogged conditions include nitrite, manganous, and others, some of which are toxic to plants (Pearsall, W.H., 1950). A brief examination was made of the effects of anaerobiosis and reduced ions on Spartina plants in culture.

IX. REACTION OF PLANTS TO ANAEROBIC CULTURE.

A. Introduction.

In the short time available, a rapid survey was made of the effect of anaerobic culture on *Spartina* plants. van Raalte (1940, 1943) showed that the rice plant maintained in its roots high oxygen concentrations, which leaked into the rhizosphere, maintaining it in an oxidized state. This had the important effect of preventing the entry of reduced ions into the roots.

Spartina roots, though stained with ferric iron in healthy sward, are unstained or blackened with ferrous iron in "die-back" sward, and clearly the rhizosphere is in a reduced state. Anaerobic cultures were begun to discover the effect of a reduced medium on growth of *Spartina*.

B. Plant cultures. 1. General Anaerobiosis.

(a) Methods. i) Plants.

Healthy *Spartina* offsets were grown in three jars which were darkened to prevent light falling on the rhizomes, yet allowed light to reach the leaves. Six rhizomes were tagged on each plant.

ii) Cultures.

One of the jars contained culture solution, and was aerated regularly. A second jar also contained culture solution, but this was covered with a layer of "Castrol" Motor Oil, and was left unaerated. (For accounts of the physical properties of motor oil, see van Raalte, M.H., 1943). The third jar contained distilled water to which had been added mud from a Spartina "die-back" pan. This was also covered with a layer of oil.

The effects of the oil were tested by growing a plant in culture under oil, and comparing it with a plant not under oil, both being aerated. Both plants grew equally well, but the aeration emulsified the oil, making manipulation difficult. Since the oil had no effect, its use was discarded in control experiments.

Measurements of the pH's and the redox potentials of the solutions were made at the beginning of the experiment. Corrections were applied to the redox potentials by the formula of Pearsall and Mortimer (1939), final values (E_7) being expressed at pH 7, (Table XX).

TABLE XX.

Anaerobic cultures - pH's and redox potentials at start.

Culture solution, Aerated.			Culture solution, Under oil.			Pan "die-back" mud, Under oil.		
pH	E_h	E_7	pH	E_h	E_7	pH	E_h	E_7
7.3	390	407	6.6	130 to 145	107 to 122	6.8	-10 to zero	-22 to -12

Observations were made on the behaviour of tillers and rhizomes, and, towards the end of the experiment, the rhizome gas contents were measured. Also watch was kept for signs of oxygen release into the culture medium around the plants.

(b) Results.

The results of the experiment are shown in Table XXI and Figure 29.

TABLE XXI.

Reaction of plants to one month's culture.

	Culture solution, Aerated.	Culture solution, Under oil.	Pan "die-back" mud, Under oil.
"Die-back" Soft rotted rhizomes (of 6	1	6	4[x]
Soft rotted tillers.	0	4	4
Rhizome gases.			
O_2	no suitable	0.0%	1.6%
CO_2	rhizome.	12.9%	15.7%
Oxidation-Reduction state.	Red-brown roots, Fe^{+++}	Black roots, Fe^{++}, S^{--}	Black roots, Fe^{++}, S^{--}

[x] Two of the six rhizomes in this experiment were accidentally left out of the solution and failed to rot.

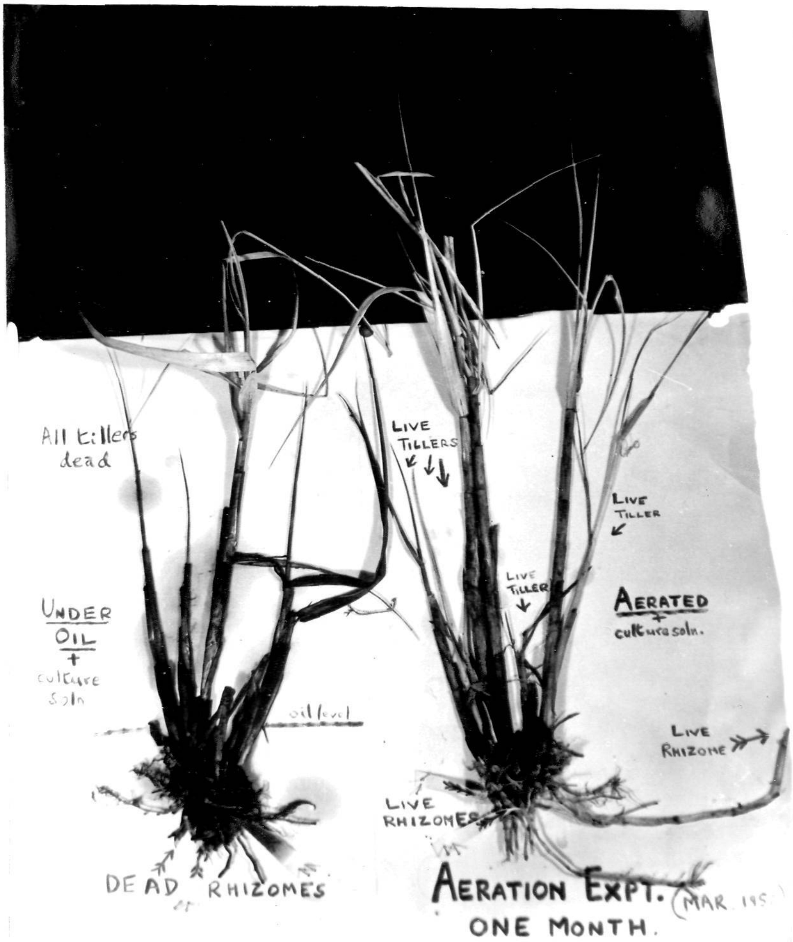


FIG. 29. Anaerobic culture of *Spartina* plants. Note soft rotting of plant in reducing conditions.

(c) Discussion.

That reducing conditions brought about plant death was evident from this experiment. All rhizomes exposed to reducing conditions died, while only one of the control rhizomes failed. In the culture with no aeration, but covered with a layer of oil, in spite of the fact that no "die-back" mud was added, the solution became reducing. This was evidently due to the presence of bacteria introduced with the plants. Sulphide bacteria are obviously present, even in healthy sward soil.

Death of the rhizomes was accompanied by a rise in the carbon dioxide content and a fall in the oxygen content. Oxygen was not transferred to the rhizosphere by the plant, as occurred in rice. This undoubtedly exposed the plant to reduced ions, in addition to the general reducing nature of the medium. No distinction could be made in this experiment between the effects of the reducing medium causing plant anaerobiosis, and any specific toxic effects of the reduced ions, of which sulphide was apparently abundant. An investigation was begun into the general effects of reducing media and the specific effects of reduced ions.

2. Sulphidic cultures.

The growth of <u>Spartina</u> in aerobic culture was compared with the growth in anaerobic culture with and without sulphides. Aerobic cultures using oxygen streams and anaerobic cultures using nitrogen streams to eliminate hydrogen sulphide were compared with ungassed, control cultures, which were both anaerobic and sulphide containing.

(a) Methods.

Healthy Spartina offsets with tagged rhizomes were grown in three darkened jars of culture solution, as in the previous experiment. One jar was supplied with a slow stream of oxygen through a 1 mm. capillary tube, a second with a stream of nitrogen, and the third, control, left ungassed.

(b) Results.

After three weeks culture the ungassed solution had blackened, and the plant was becoming weak, though still alive. The other solutions were brownish, containing no ferrous sulphide.

After six weeks the rhizomes in the control culture had begun to soft rot, but at the same time the other plants were attacked by a fungus at water level. The appearance of the fungal attack was different from that of the rhizome rot, but quite invalidated the experiment.

(c) Discussion.

It was necessary to design new experiments to prevent attack by fungi in gassed cultures. Further experiments, aimed at excluding fungi, were carried out.

C. Rhizome cultures.

Immediate submerged culture of rhizomes from healthy plants in the field eliminated fungal attack at water level. Such cultures were liable to bacterial attack if aerated, but this was prevented by penicillin doses of 500 units/cc. culture solution/week. No bacterial attack occurred in reducing conditions.

First trials showed that the gas culture techniques used on the whole offsets in the large jars were no longer suitable in the Ehrlenmeyer flasks used for the rhizome cultures. The rhizomes brought insufficient sulphur bacteria into the culture to make it reducing, and the penicillin also prevented this. Addition of pan "die-back" mud was also unsuccessful, partly on account of the elimination of the bacteria by penicillin, and partly owing to the fact that rhizomes would occasionally be buried under the mud, owing to inefficient stirring by the gas streams. Rhizomes thus buried became anaerobic and died. Since other methods of introducing sulphide into the cultures failed, experiments were made on the effects of supplying inorganic sulphide reagents.

1. Methods.

Four strong rhizomes were grown in each of four Ehrlenmeyer flasks in a light-tight box. Two contained 100 ml. of glass distilled water, the other two, 100 ml. of

1/10th strength Knop culture solution with a trace of manganese. To one flask of water, and one of culture solution, was added the equivalent of 3.0×10^{-3}g.% S^{--}, as sodium sulphide, a similar level to that found in "die-back" soils (Section VIII). Each solution also contained a total of 50,000 units of penicillin (sodium salt).

After one week's culture, with all the rhizomes growing strongly, a further 100 ml. normal Knop culture solution was added to the culture solution flasks, and 100 ml. glass distilled water to the water flasks. A further equivalent of 3.0×10^{-3}g.% S^{--} was added, and another 50,000 units of penicillin. With the substitution of normal Knop solution for the 1/10 strength used in the previous week, a noticeable black precipitate was obtained from the iron in the solution to which sulphide was added.

The cultures were examined after a further week (i.e., 2 weeks after setting up).

2. Results.

Soft rotting near the apex occurred in all the rhizomes in the culture solution to which sulphide had been added (Fig. 30). The failure bore a marked resemblance to that found in "die-back" areas (Fig. 1). All the rhizomes in the other cultures (including that in which sulphide had been added to distilled water) were

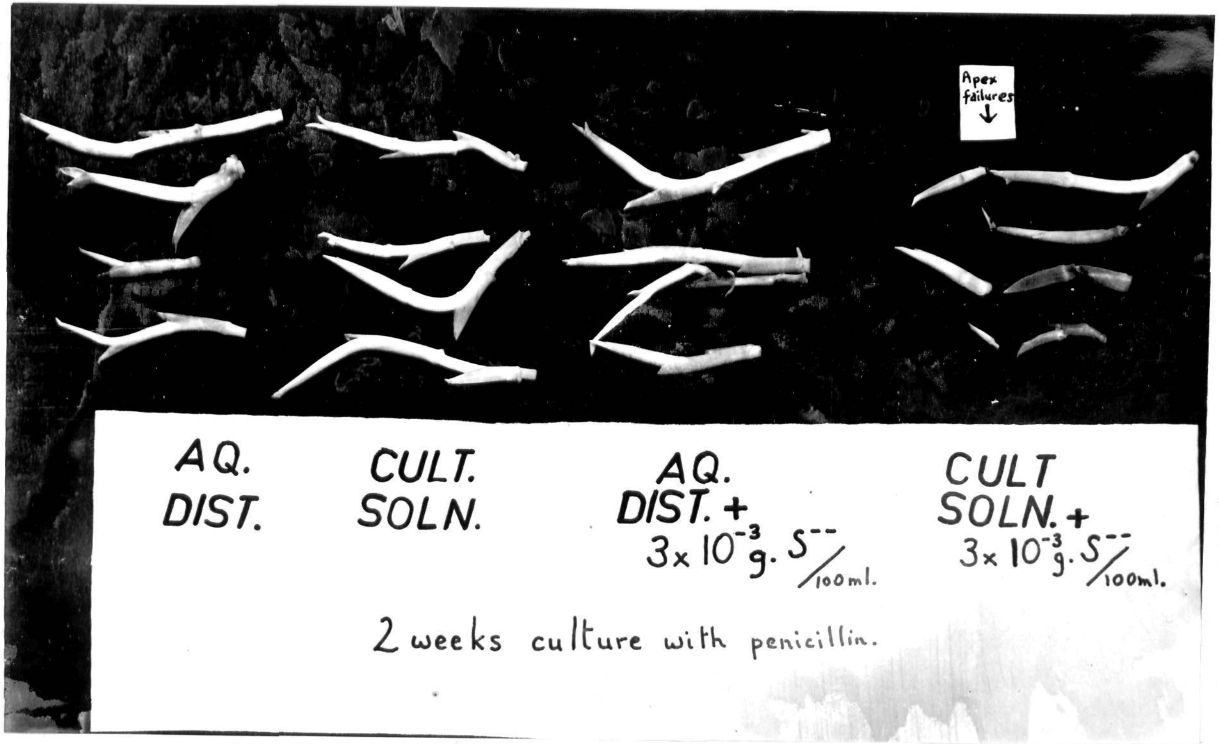


FIG. 30. Sulphidic culture of *Spartina* rhizomes. Note the soft rotting in ferrous sulphide culture only. (cf. Fig. 1).

growing strongly. A test for sulphide in the distilled water-sulphide flask showed that the sulphide had disappeared completely. In the culture solution-sulphide flask the black colour showed that ferrous sulphide was still present.

3. Discussion.

Rhizome apex failure appeared in response to the presence of ferrous sulphide, and this result is regarded as of prime importance. In the absence of iron, sulphides are quickly eliminated from cultures, as in the distilled water-sulphide flask. When iron is present, however, as in the culture solution-sulphide flask, ferrous sulphide acts as a "reservoir" maintaining the culture saturated with ferrous sulphide. There is a remarkable similarity between this system and that suspected in soils (Section VIII).

As in soils, the presence of ferrous sulphide in cultures implies not only the existence of Fe^{++} and S^{--} ions, but that of Mn^{++} and NO_2^- and of a generally reduced state throughout the medium. It is not possible in this type of experiment to single out the toxic factor, but there can be no doubt that it is based on the reduced complex controlled by ferrous sulphide, if it is not the ferrous sulphide itself.

X. DISCUSSION.

A. The Problem.

Massive invasions by plants have seldom been recorded in our time on such a scale as that of *Spartina townsendii*. Indeed, comparisons have been made with the spread of *Opuntia*, *Eichhornia* and *Elodea* (Oliver, F.W., 1920). Undiscovered a century ago, *Spartina townsendii* is now the commonest higher plant of the sea shore, having covered thousands of acres of intertidal land in a single human lifetime. It is the more surprising that such a vast extension has had so few economic repercussions, but this is largely due to the fact that the land occupied was, in our own country, of little value. Elsewhere, the extension has been of some importance in reclaiming land from the sea.

Yet it seems that already - even twenty years ago - the tide of the invasion of new land by *Spartina* was being turned, as happened after a time with *Elodea*, and as may be expected of plants that suddenly extend their range. Unlike other plants, though, the invasion by *Spartina* was so well recorded by earlier botanists that the history of the plant can be followed in great detail, both in the phase of extension, and in the early stages of recession as we see them today. This recession - "die-back" - seems likely to reduce *Spartina* to a new, but less important equilibrium position among the plants of the sea shore, and its mechanism is accordingly of great interest.

Recession of such an aggressive invader as *Spartina* has not only academic interest, but cannot fail to have some few practical and economic aspects. The dredging problem at Lymington appears to be the first of these. Also, future use of *Spartina* for land reclamation and coastal protection will have to take into account the possibility of later "die-back". Thus, "die-back" must be considered both in the light of academic and practical interests.

B. Fundamental Aspects.

Investigation of such a general phenomenon as "die-back" has necessitated studies in a wide range of fields, but three of these have revealed fundamental aspects of particular interest. These are:-

1. The Ecology and Physiography of the Spartinetum.
2. The Physiology of Growth of *Spartina*.
3. The Chemistry of the *Spartina* soil.

1. The Ecology and Physiography of the Spartinetum.

Salt marshes are situated on tidal flats on the coasts of England, Scotland and Wales, in harbours, estuaries, rivermouths and the lees of shingle banks, but rarely exposed to the open sea. The salt marsh substrates vary between the different coasts of our country. On the west coast the substrates are very sandy, and mobile, being capable of fast accretion, yet easily swept away by storm tides. On the east coast, in the north are shingle barred marshes with shingle and sand behind the bars, but firm silt or clay towards the mainland; in the south are estuarine marshes, with fine silt and clay substrates. On the south coast the marshes are mainly estuarine, though occasionally shingle barred. They are almost invariably sited on fine river silts and clays, with practically no sand, and hence their draining properties are very poor.

It was the salt marshes - some with other plants of the general salt marsh community already established - that Spartina invaded, in some cases by natural means, as often by planting. The most recent spread has been to the marshes of the west coast which are, as yet, not greatly altered in appearance, though the plant is growing vigorously in some western areas. Dense and strongly growing swards are beginning to form where river silt sweeps over the marshes, but these marshes are today little more than extensions of the existing Puccinellia marshes, and the Spartina marsh has little structure of its own.

On the east coast, in the north where the grass has been established longest it is in dense sward, though there is no distinct marsh structure, but merely local variations in Spartina growth resulting from outcrops of sand and shingle; in the south, on the silty estuarine east coast marshes, Spartina is accreting much river sediment and building marsh compartments separated by drainage channels. In some compartments of the marsh at Mistley in the Essex Stour areas of particularly wet silt are forming, and it seems that waterlogging of the soil is commencing.

On the south coast where the silt is particularly fine, Spartina has grown for the longest period, and the silt accreted by the plant lies thickest. The compartments of the marshes here have a distinct structure of their own, evidently achieved by differential accretion. More, firmer, silt seems to be accreted by the Spartina stems beside both major and minor drainage channels of the compartments, forming "levées", while between the channels less, wetter, silt settles. Although the compartments in general rise towards the centre, the levées impede lateral drainage, forming, towards the periphery, "incipient pans", and in the centre of the compartments fully waterlogged "pans". Thus successive, increasingly waterlogged pans are found whose level rises away from the main drainage channels, and beside each pan are levées, on which the Spartina grows healthily, separating the pans from the minor channels. The levées are occasionally breached by the force of the ebb tide, when "gullies" form, and the pans drain out. When this happens "butts" of turf may be delimited by the gullies, and these butts often slip into the nearby channel, effecting pan breakdown. By pan formation and breakdown extensive damage may occur to the sward. Pan processes have been occurring most markedly at Lymington since 1924, and are still active.

In addition to the accentuation of the poor drainage of the southern silts in the sward by pan formation, another factor comes into play beside the main channels of the marshes, at a low level (more than 6 hrs. immersion on the high tide) on the edge of the sward. This factor results in accretion of a similar wet silt and clay to that in pan areas, and is especially noticeable on the north eastern banks of the estuaries. The nature of the factor is unknown, but its effect in the north east suggests that it may be governed in some way by the prevailing south westerly winds. It seems possible that the winds might drive the bulk of the fresh water outflow of the river overlying the saline tide across the north eastern sward depositing more of the fine silt in that area than elsewhere. Whatever the cause, fine particled soil and waterlogging result. Waterlogging apparently became acute along the channel side at Lymington in 1935.

Waterlogging is thus characteristic of fine silt and clay substrates where the poor drainage is accentuated by lateral impedance resulting from levée formation in the compartments or by the factor at work on the main channel bank. Since both these factors have been seen at work in all the oldest silt and clay substrated swards, apparently every such sward is liable to eventual waterlogging. Since the grass itself accretes a fine silt, it may be that if the supply of coarser material is cut off in other areas by any chance, these swards, too, may be converted to fine silt and clay soils, and waterlogging will follow.

The development of the swards, and their eventual waterlogging, make a particularly interesting study in ecology and physiography. Sward development evidently depends on several factors:-

(a) The original substrate, before invasion by Spartina.
This will depend on i) Past supply of material,
and ii) Past distribution of material.

(b) The present regime of accretion, under Spartina.
This has two aspects: i) Gross supply and distribution - formation of the massive structure of the marsh - drainage patterns and marsh compartments,
and, ii) Local supply and distribution - formation of the minor structure of the marsh - levée patterns and distribution of different particles within the compartments, and the resultant waterlogging.

(c) The special case of channel side accretion, the distribution of material at the edge of the marsh, and the resultant waterlogging.

Ecological and physiographical studies are being conducted by Miss E. M. Uren with the object of more closely defining the systems outlined in this preliminary work, and studying the developmental processes of Spartineta as a whole.

2. The physiology of growth of Spartina.

"Die-back" of plants has consistently been associated with waterlogged sites. Transferring healthy plants into the waterlogged "die-back" sites or into "die-back" soils in the laboratory caused them to die, while dying plants from the edge of "die-back" sites recovered when transferred to the unwaterlogged, healthy sward, or to well drained laboratory culture. Variations in Spartina growth occur on a variety of substrates - dwarfing results from growth on sand and shingle and where there is strong wave erosion, but only in the waterlogged "die-back" sites does death follow.

"Die-back" is independent of pathogenic organism attack as shown by infection experiments, and independent of mineral unbalance, as shown by chemical analyses of the plants and soils. Neither is "die-back" associated with pollution of the soil by chemical substances though this has, in the past, occasionally caused death of plants locally.

Study of the development of Spartina has shown that buds are formed during the winter, and grow in the spring, the shoots lengthening and flowering in the long summer days (though whether this is a true photoperiodic effect, or merely one of light quantity is not known). Shoots grew tallest in the healthy sward, and flowering was most vigorous and longest maintained there, healthy sward productivity always being greater than that of "die-back" sward. In November buds were laid down in all sites, but many more buds formed in healthy sward than in "die-back" sward. These buds formed both tillers and rhizomes, and during the winter months rhizome growth was especially strong in the healthy sward. In the "die-back" sward rhizome deaths occurred throughout the winter, the apices of the rhizomes becoming feeble and often being replaced by buds from the axils of the scale leaves. The primary stages of the rhizome "soft rot" were not associated with pathogen attack, though secondary bacterial infection followed, to achieve the final breakdown of the rhizome, leaving only the tougher cortical and vascular elements. By early spring when flowering had finished, the rhizomes had upturned, and the tiller buds were clustered in the axils of the leaves of the short rosette shoots. At this time of year the number of buds which had overwintered was far greater in the healthy sward than in the "die-back" sites. In "die-back" sites the shoot population was drastically reduced each spring by the production of fewer buds, and by the failure of many of these to survive the winter, both as tillers and as rhizomes, and by the failure of rhizomes to turn up. Together these factors reduce the vitality of the "die-back" sward plants so that the proportion of young parts becomes less year by year, and the plants become moribund, and eventually die.

It is rhizome production that effects the most vigorous vegetative reproduction, as these organs produce shoot clusters at a distance from the parent, while tillers produce one, or occasionally more, daughter shoots near the parent stock. Failure of the rhizomes is thus especially serious in its consequences to the plants. Examination of the rhizomes showed that their internal space diaphragms were highly efficient, rendering the passage of gases through the rhizome difficult. The spaces contained very little oxygen and very much carbon dioxide in "die-back" sites, and alcohol was present, while healthy sward rhizomes had higher oxygen contents but less CO_2 and alcohol. The low oxygen content of "die-back" rhizomes was ascribed to the reducing nature of the "die-back" soil (see below), and it was considered likely that the carbon dioxide levels encountered in "die-back" rhizomes would be sufficient to cause narcosis and possibly death of the plants in these conditions.

This central aspect of Spartina growth - the physiology of growth in healthy sward and "die-back" conditions - appears to be outlined in fair detail, and it seems unlikely that the field can be greatly extended at present. A pure study of the physiology of growth of the plant could be continued, but the plant has difficult habitat requirements for either field or laboratory investigation of normal growth physiology, and more academic studies of the plant would accordingly be complicated.

3. Chemistry of the Spartina soil.

It is believed that waterlogging is directly responsible for the initial Spartina deterioration in "die-back" sites, and this must return organic matter to the soil. Important changes follow. The increased organic matter causes a rise in the soil water content far above that already held by the fine silts of the waterlogged areas, and thus the waterlogging and "die-back" are self-regenerating. In addition to speeding the "die-back" and waterlogging, the presence of organic matter leads to more fundamental changes in the soil composition.

Even healthy Spartina sward soils contain abundant bacteria (Clitheroe, A., 1955), and in the waterlogged "die-back" sites the increased organic matter and water contents must lead to a great increase in the activity of facultative and true anaerobes, and the soil oxygen must be exhausted rapidly. No recharging of the soil oxygen occurs between tides, as the water of composition of the finely particled silts is not replaced. The waterlogged soils become stagnant, and the oxygen content falls to zero, with reduction of all the other ions present. Ferrous ions are produced from ferric, manganous from manganic, and sulphide from sulphate.

Although in some other waterlogged soils leaching of ferrous ions occurs, as they are more soluble than ferric ions, the stagnant nature of the *Spartina* soil evidently prevents this. Even where the plants have died back for a considerable period of time, the soils seem to have undergone little change, and this may be due to the cold climate causing less activity in the highly organic sediment than occurs, for instance, in rice soils (Pearsall, W.H., 1950). The reduced complex based on ferrous sulphide seems to be present abundantly and to be quite stable in the waterlogged "die-back" *Spartina* soils.

Tests on soils and on cultures show that under these circumstances the ferrous sulphide "reservoir" maintains the solution saturated with ferrous sulphide, and much is thus available to plants.

Unfortunately with present techniques it is not possible to distinguish the reducing effects of the ferrous sulphide and its toxicity. It seems possible that the reducing effects alone could harm the Spartina plant (Section VII above), if not kill it, since it is poorly equipped to withstand reducing conditions, but even if this were not so it seems likely that the toxic nitrite, manganous, and especially the abundantly available ferrous and sulphide ions would be sufficient to kill the plant. Together, the highly reduced medium and the toxic ions effect quick plant death in culture and almost certainly also in the habitat.

There are clearly two separate, but closely related phases in the development of the reduced soil. The actual reduction of the soil is caused by the bacteria present, and though a preliminary investigation was made by Miss A. Clitheroe, more detailed studies could be pursued on the bacterial flora of the marine muds.

The exhaustion of oxygen in the soil by bacteria leads to the production of reduced ions. The Spartina soil is a new field for the investigation of the effect of waterlogging on soil minerals. The soil is of interest as it has a high unleached content of both organic and inorganic substances, of which ferrous sulphide is especially important.

By far the most interesting field, however, is that of the relations of the *Spartina* plant to the waterlogged soil conditions. There are few such soils which support plant life, and the one most studied, that of the rice plant, is more often leached than ion rich. The relation between the *Spartina* plant and the reduced ions of the waterlogged soils - their uptake and effects on the plant - could be the subject of an important and informative fundamental study.

C. Practical Aspects.

1. Shipping and Dredging.

The extension of *Spartina* stabilized and extended mud banks in the estuaries, rivermouths, and harbours around our coastline, but apparently caused only minor inconvenience to yachtsmen. In areas with free access of the tide, the rivers were narrowed, but the scour must have remained constant, so that the dredging requirements might have been reduced. In areas with restricted access of the tide Oliver (1925) predicted that the reduced scour occasioned by the narrowing might cause increased dredging requirements. In the phase of extension of *Spartina* no increase was observed in the dredging requirements in the restricted harbour at Poole, and as far as is known the dredging requirements in the rather open sward at Lymington do not appear to have been greatly reduced.

Recession of the grass might be expected to have the opposite effects to extension, though if the recession was rapid, it would cause silting under all conditions. Although the Poole dredging requirements do not seem to have altered as a result of the slight Spartina recession in that area, those at Lymington seem to have increased. Large amounts of material being returned to a rather narrow river such as that at Lymington, in a short time, would necessarily have the most severe effects. On examination, however, the effects at Lymington are not as serious as was originally thought. Other rivers on the south coast do not require such thorough dredging as the Lymington river, even in normal times, and they are generally wider, and their Spartineta less extensive, so that recession in these areas is unlikely to give rise to serious problems.

2. Land Reclamation and Coastal Protection.

During the phase of extension Spartina found use as an agent for land reclamation between the tide marks, and for coastal protection in front of sea walls. The first trials met with varying success. Where the intertidal land was sheltered - in estuaries, harbours, and rivermouths - and the substrate was a sandy silt, or a silty clay, growth was successful (Oliver, F.W., 1920; Bryce, J., 1931; Yapp, R.H., 1923). In less sheltered areas (Roper, I., 1922), and where the substrate was very sandy (Konig, D., 1949), growth was not successful.

Considerable extension of the flats covered by Spartina has taken place, and an appreciable amount of land has been raised from the intertidal zone to near the high tide mark. Unfortunately, this land still receives regular tidal inundations, as Spartina is rarely replaced by other plants. On sandy silts where the drainage is good, as on the west coast, little harm seems to result from leaving the Spartineta unmanaged, as the sward provides acceptable grazing for sheep, if required, and this may even encourage succession to Puccinellietum. No deterioration occurs in swards left entirely in their natural state.

By contrast, on wet silty clays the Spartineta, having been raised to near the high tide mark, undergo "die-back" and deteriorate rapidly. The swards have no value for grazing, as they are easily penetrated by animal hooves, and ponies alone graze them, and then only at the landward margins. Pony grazing encourages no replacement of the Spartina by other plants, and is even damaging to the sward, as hooves puddle it. In these sites succession seems only to lead to poor, waterlogged Spartinetum.

Waterlogging of the swards gives way to progressive "die-back" which reduces them to areas of stagnant mud with a feeble growth of plants of them. From time to time the mud is cut back into the river channels by the tide. This is the state that has been reached in the Lymington river, and it seems only a question of time before it becomes general throughout the wet south coast Spartineta.

To prevent complete degeneration of the Spartineta on the south coast, it might be possible to carry out poldering or similar operations, but these would be expensive, particularly on the long winding river channels. Such methods have been used in Holland, it is believed both with and without the use of Spartina, but they have been confined to reclamation of squares by constructing polders and extending them one side at a time until the area is reclaimed completely. Such reclamation is likely to prove as expensive when Spartina is used as when it is not, though the plant may possibly make work easier. In Holland, though, land is valuable, and the reclamation work is soon repaid, whereas in England it is doubtful whether the high cost of reclamation could ever be recovered.

While reclamation work would almost certainly be unprofitable on silts and clays, sandy silts offer great possibilities. Such land provides useful grazing, and with proper management this might even achieve replacement of the *Spartina* by more nutritive grasses. The land is firmer, and more easily worked, should this be necessary. Reclaiming would probably not be difficult, and study of the possibilities of sandy silt reclamation on the west coast might prove profitable agriculturally.

XI. ACKNOWLEDGEMENTS.

My most sincere thanks are due to Professor W. T. Williams for his kind advice and guidance throughout this work, and also to Dr. J. M. Lambert and Dr. J. G. Manners of the Department of Botany at Southampton for their helpful advice.

The work was made possible by a Research Assistantship provided by the Agricultural Research Council, whose generosity it is a pleasure to acknowledge.

I wish to record my thanks to Sir Edward Salisbury for his permission to consult the Staff and records of the Royal Botanic Gardens, Kew. Mr. Peter Austwick, who prepared a preliminary report on Spartina "die-back" for the Director, and Mr. C. E. Hubbard, have both given valuable advice.

A considerable debt of gratitude is owed by me to the members of the Academic, Laboratory, and Garden Staff, and also to certain students of the Department of Botany at Southampton for their advice, co-operation, and practical assistance. Also I would thank the Academic and Laboratory Staffs of the Departments of Chemistry, Zoology, and Physiology for advice and loan of apparatus.

For advice, and, in several cases, for practical assistance, I wish to thank Professor S. Mangham of Southampton, Dr. I. Wilson and Mr. E.H. Chater of Aberystwyth, Mr. H.H. LeRiche of Rothamsted, Dr. Rutter of Imperial College, Mr. D.A. Coult of Liverpool, and others mentioned in this work.

For advice and loan of maps, dredging plans, and boats in the early stages of the investigation I have to thank Mr. Franks, Hydrographic Survey Officer of the Docks and Inland Waterways, his and other Staff of that Department, and especially those stationed at Lymington.

For permission to carry out certain investigations at Lymington, and for assistance during their course, I have to thank the Harbour Commissioners, and Mr. R. Conway. Many other citizens of the Borough, and elsewhere, have provided valuable information and records of Spartina.

I am grateful to those persons who have lent photographs for study. The co-operation of the Staffs of the Ministry of Town and Country Planning, and of Aerofilms Ltd., and especially of Dr. J.K. St. Joseph of the University of Cambridge has been a great help in Spartina surveys.

I would acknowledge the kind co-operation of the Officers of the Nature Conservancy Research Station, Furzebrook, and of Mrs. P-A. Caldwell, recently of University College, London, whose studies in related

fields have provided valuable information.

Mr. F.A. Barrett has exercised considerable skill in photographing difficult material and diagrams, and I am greatly indebted to him. Mrs. Margaret Phillips I thank for her skill and accuracy in typing this thesis.

Finally, it is a great pleasure to acknowledge the helpful co-operation of Miss Elspeth Uren who joined this research in July 1955 to carry out studies on the more ecological aspects of the problem and to whose further work I wish all success.

XII. REFERENCES.

ALLAN, H., (1931). Spartina townsendii. N.Z.J. Agric. 42, 295-299.

AUSTWICK, P.K.C. (1950). Report on investigations into the cause of dying out of S. townsendii in Lymington Harbour.
Report to the Director, Royal Botanic Gardens, Kew. (Abstracted by permission of Sir. E. Salisbury and Mr. Austwick).

BARGHOORN, E.S. and LINDER, D.H. (1944). Marine fungi: their taxonomy and biology. Farlowia. 1, 395-467.

BARTON, C.J. (1948). Photometric analysis of Phosphate rock. Analyt. Chem. 20, 1068-1073.

BENEDICT, H.M. (1941). The inhibitory effect of dead roots on the growth of brome grass.
J.Amer.Soc.Agron. 33, 1108-1109.

BERGMANN, H.F. (1920). The relation of aeration to the growth and activity of roots and its influence on the ecesis of plants in swamps.
Ann.Bot., Lond. 133, 13-33.

BONNER, J. (1950). The role of toxic substances in the interactions of higher plants.
Bot. Rev. 16, 51-65.

BRACHER, R. (1933). S. townsendii on the Portishead salt marshes. J. Bot., Lond. 71, 233-234.

BRYCE, J., (1931). Further notes on economic possibilities of Rice Grass.
J. Minist. Agric. 38, 801-805.

_______ (1941). The economic exploitation of Rice Grass.
Emp.J.exp.Agric. 9, 167-170.

BUTCHER, R.W. (1935). Wasting disease of Zostera marina.
Nature, Lond. 135, 545.

CAREY, A.E. and OLIVER, F.W. (1918). Tidal Lands. pp. 176-184. London: Blackie.

CHAPMAN, V.J. (1941). Studies in Salt Marsh Ecology VIII.
J.Ecol. 29, 69-82.

CLITHEROE, A. (1955). Report on the investigation of the bacteria from mud in Lymington River.
Undergraduate Thesis, University of Southampton.

CONWAY, V.M. (1937). Studies in the autoecology of Cladium mariscus III. Aeration of subterranean parts of the plant. New Phytol. 36, 64-96.

COOK, W.R.I. (1926). The Genus Ligniera.
Trans.Brit.mycol.Soc. 11, 196-231.

COSMO-MELVILLE, J. (1906). Spartina townsendii.
J. Bot., Lond. 44, 356.

ELLIS, J.B. and EVERHART, B.M. (1885). **Leptosphaeria spartinae** E. & E. J. Mycol. 1, 42-44.

ELLIS, J.B. and TRACY, S.M. (1890). *Uredo peridermiospora*. J. Mycol. *6*, 76-77.

GERRITZ, H.W. (1935). Digesting Biological materials for Calcium and Phosphorus analysis. Analyt.Chem. *7*, 167-168.

GLASSTONE, S. (1951). Textbook of Physical Chemistry, 2nd ed., pp. 1077-1078. London: Macmillan.

GOODALL, D.W. and GREGORY, F.G. (1947). Chemical composition of plants as an index of their nutritional status. p. 167. Aberystwyth: Imperial Bureau of Horticultural and Plantation crops.

GREGORY, F.G. and PEARSE, H.L. (1934). The resistance porometer and its application to the study of stomatal movement. Proc.roy.Soc. B. *64*, 477-493.

GROVE, W.B. (1933). *Didymosphaeria spartinae*. J.Bot., Lond. *71*, 259.

__________ (1937). British stem and leaf fungi. 2 vols. Cambridge: University Press.

GROVES, H. & J. (1881). On *Spartina townsendii* Groves. Rep. bot.(Soc.) Exch.Cl.Manchr. *8*, 37.

GUSTAFSON, F.G. (1934). Production of alcohol and acetaldehyde by tomatoes. Plant Physiol. *9*, 359-369.

HUMPHRIES, E.C. (1956). in Paech, K. and Tracey, M.V. Modern methods of plant analysis. Vol. 1. pp.468-502. Berlin: Springer-Verlag.

JACQUET, J. (1949). Recherches Écologiques sur le Littoral de la Manche. Encyclopédie Biogeographique et Écologique. Paris: Lechevalier.

JØRGENSEN, C.A. (1934). Plantnings forsøg Med <u>S. townsendii.</u> Bot. Tidsskr. <u>42</u>, 420-440.

KNOWLES, F. (1929). The Economic Possibilities of Rice Grass III. Composition and Nutritive value. J. Minist. Agric. <u>35</u>, 934-939.

KONIG, D. (1949). Die Ausbrietung des Grases <u>S. townsendii</u> schleswigholsteinischen Westküste. Forsch. Fortschr.dtsch.Wiss. <u>25</u>, 84-87.

LAING, H.E. (1940). Composition of the internal atmosphere of <u>Nuphar</u> and other water plants. Amer. J. Bot. <u>27</u>, 861-868.

LANGER, R.H.M. (1956). Growth and nutrition of **Timothy.** I The life history of individual tillers. Ann. appl. Biol. <u>44</u>, 166-187.

LINTON, E. (1906). Spread of <u>S. townsendii.</u> J.Bot., Lond. <u>44</u>, 392.

LLOYD, L.S. (1952). An investigation of the fungi of

S. townsendii from the Dovey estuary. Undergraduate Thesis, University of Wales, Aberystwyth.

McDONALD, M.E. (1955). Causes and effects of a die-off of emergent vegetation. J. Wildlife Mgmt. 19, 24-35.

McPHERSON, D.C. (1939). Cortical air spaces in the roots of Zea Mays L. New Phytol. 38, 190-202.

MONTAGU of Bealieu (1907). in Royal Commission on coastal erosion, Minutes of Evidence. Vol. 1, pp. 290-300. Vol. 2, pp. 311-360. London: H.M.S.O.

MORTIMER, C.H. (1941). The exchange of dissolved substances between Mud and Water in Lakes. I and II. J. Ecol. 29, 280-329.

________ (1942). The exchange of dissolved substances between Mud and Water in Lakes. III & IV. J. Ecol. 30, 147-201.

MOUNCE, I. and DIEHL, W.M. (1934). A new Ophiobolus on Eel grass. Canad. J. Res. 11, 242-246.

NELSON, A. and MUNRO, J. (1934). The germination of S. townsendii. Notes of Roy.Bot.Garden, Edinburgh. 18, 173-180.

OLIVER, F.W. (1920). Spartina problems. Ann.appl.Biol. 7, 25-39.

________ (1925). S. townsendii; its mode of establishment, economic uses and taxonomic status. J.Ecol. 13, 74-91.

PEARSALL, W.H. (1938). The soil complex in relation to plant communities. I. Oxidation-reduction potentials in soils. J.Ecol. 26, 180-193.

__________ (1950). The investigation of Wet soils and its Agricultural Implications. Emp.J.exp.Agric. 18, 289-298.

__________ and MORTIMER, C.H. (1939). Oxidation-reduction potentials in Waterlogged soils, Natural waters, and Muds.I and II. J.Ecol. 27, 483-501.

PERRATON, C.J. (1953). Salt marshes of the Hampshire-Sussex border. J.Ecol. 41, 240-247.

PHILLIPS, M.E. (1953). Studies in the quantitative morphology and ecology of Eriophorum Roth. J. Ecol. 41, 295-318.

PIPER, C.S. (1950). Soil and Plant analysis. pp. 362. University of Adelaide.

PIRSON, A. (1955). Functional aspects in Mineral Nutrition of Green plants. Ann.Rev.Plant Physiol. 6, 71-114.

POWELL, F.J.N. (1953). The determination of Calcium in Biological fluids by Flame Photometry. Journal of Clinical Pathology 1953, cited by Evans Electroselenium Ltd. in Flame Photometer Data Sheet D3.

PRATT, M.C. (1953). S. townsendii on the Dee marshes. N-W.Nat. (NS). 1, 98-99.

PURVIS, O.N. (1934). An analysis of the influence of temperature during germination on the subsequent development of certain winter cereals and its relation to the effect of length of day. Ann.Bot., Lond. 48, 919-955.

ROPER, I. (1922). Spartina and coast erosion. Kew Bull. 5, 351-352.

RUSSELL, R. Scott, (1954). The relationship between metabolism and the accumulation of ions by plants. Symposia of the Society of Experimental Biology, Part 8; Active Secretion and Transport. 343-366.

SACCARDO, P.A. (1898). Sylloge fungorum: Host Index: Vol. 13, pp. 1-1340. (Lithoprinted 1944): Michigan: Edwards.

SCHOLANDER, P.F., van DAM, L., and SCHOLANDER, S.I. (1955). Gas exchange in the roots of mangroves. Amer. J. Bot. 42, 92-98.

SKELDING, A.D., and WINTERBOTHAM, J. (1939). The structure and development of hydathodes of S. townsendii. New Phytol. 38, 69-79.

SNELL, F.D., and SNELL, C.T. (1949). Colorimetric methods of analysis. 3rd Ed. Vol. 2, pp. 748. New York: Nostrand.

STAPF, O. (1908). Spartina townsendii. Gardeners' Chronicle; 1, 099; 33-35.

SUTHERLAND, G.H. and EASTWOOD, A. (1916). The physiological Anatomy of S. townsendii. Ann.Bot., Lond. 30, 333-351.

SWANN, E.L. (1950). New Land from Grass. Countryside (NS) 15, 167-169.

UNESCO (1954). Arid Zone Programme IV. Reviews of Research on Problems of Utilization of Saline Water. pp. 96. Paris: UNESCO.

VALLANCE, K.B. and COULT, D.A. (1951). Observations on gaseous exchange between Menyanthes trifoliata and its environment. I. Composition of the internal gas in the plant. J.exp.Bot. 2, 212-222.

VAN RAALTE, M.H. (1940). On the oxygen supply of Rice roots. Annales du Jardin Botanique de Buitenzorg. 50, 101-114.

__________ (1943). On the oxidation of the environment by the roots of Rice. Syokubutu-Iho. 1, 2603, 15-34.

VOGEL, A.I. (1944). A textbook of Quantitative Inorganic Analysis. pp. 856. London: Longmans.

WEBB, D.A. (1939). Observations on the blood of certain Ascidians, with special reference to the biochemistry of Vanadium. J.exp.Biol. 16, 499-523.

YAPP, R.H. (1923). S. townsendii on the Dovey salt marshes. J.Ecol. 11, 102.

APPENDIX 1.

Index to principal photographs of Spartineta used in surveys.

Site.	Date.	No. & Type.	Photographer.
Lymington.	1904	1 Ground	Miss Bale.
Havant.	1911-'26.	7 Ground	Prof. S. Mangham
Poole.	1920	7 Ground	Mr. W. Woodhouse.
Newtown, I.O.W.	1923	8 Ground	Prof. S. Mangham.
Hythe-Hurst.	1923-'26.	76 Vertical aerial	R.A.F., prints only, in So'ton.
Havant	1923-'26.	20 Vertical aerial	
Totton, So'ton.	1924	3 Ground (pollution)	Prof. S. Mangham.
Eling, So'ton.	1925-'27.	7 Ground (S. alterniflora)	Prof. S. Mangham.
Lymington.	1925-'35.	5 Ground	Mr. Rand.
Lymington.	1934	1 Oblique aerial	Aerofilms Ltd.
So'ton Water.	1945-'55.	6 Oblique aerial	Aerofilms Ltd.
Beaulieu.	1946	Complete mosaic	R.A.F.
Lymington	"	" "	"
Essex Stour.	"	" "	"
Blakeney.	"	" "	"
Havant.	"	" "	"
Beaulieu.	1948	1 Oblique aerial	Dr. J.K.St.Joseph.
Lymington.	1948	2 Oblique aerial	Dr. J.K.St.Joseph.
Keyhaven.	1949	1 Oblique aerial	Dr. J.K.St.Joseph.
Lymington.	1949	1 Oblique aerial	Aerofilms Ltd.
Christchurch.	1950	1 Oblique aerial	Aerofilms Ltd.
Lymington.	1951	3 Vertical aerial	R.A.F.

Poole.	1952	1 Oblique aerial	Dr. J.K.St.Joseph.
Lymington.	1952	1 Oblique aerial	Aerofilms Ltd.
Beaulieu.	1954	16 Oblique aerial	Dr. J.K.St.Joseph.
Poole.	1954	16 Oblique aerial	Dr. J.K.St.Joseph.
Havant.	1955	7 Ground ('11-'26 rephotographed)	Mr. C.J. Perraton.
Keyhaven.	1955	12 Oblique aerial	Dr. J.K.St.Joseph.

b. Names and Addresses of Photographers.

i) The Misses Bale,
High Street,
Lymington, Hants.

ii) Professor S. Mangham,
c/o Botany Department,
The University,
Southampton, Hants.

iii) W. Woodhouse, Esq.,
Twynham Road,
Southbourne,
Bournemouth, Hants.

iv) Mr. Rand,
Avenue Road,
Lymington, Hants.

v) Aerofilms Ltd.,
29, Old Bond Street,
London, W.1.

vi) Dr. J.K. St. Joseph,
Curator in Aerial Photography,
Sidgwick Avenue,
Cambridge.

vii) C. J. Perraton, Esq.,
60, East Street,
Havant, Hants.

Copies of certain photographs, in some cases where only single copies are known to exist, are available for inspection in the Botany Department, the University, Southampton.

APPENDIX 2.

Origins and Dates of Establishment of Principal Spartineta.

South Coast Marshes.

Site.	Origin.	Date.	Authority.
Hythe.	?	1870	Sutherland, G. & Eastwood, A., 1916.
Yarmouth, I.O.W.	a	1893	Stapf, O., 1908.
W.Medina, I.O.W.	a	1895	Stapf, O., 1908.
Lymington.	a	ca. 1896	Cosmo Melville, J; Linton, E. 1906.
Beaulieu.	p	1898	Mills, D. in litt. 1931.
Chichester.	a	1899	Stapf, O., 1908.
Poole Harbour.	a	1899	" "
Havant.	a	1900	" "
Bosham.	a	1901	" "
Keyhaven.	a	1903	" "
Hamble.	a	1907	" "
Wootton, I.O.W.	a	1907	" "
Fawley.	a	1907	" "
Christchurch.	a	1913	Oliver, F.W., 1925.
Pagham.	a	1918	Oliver, F.W., 1925.
Rye.	a	1922	Oliver, F.W., 1925.
Dawlish.	a	1930	Spooner, R. verbal communication.
Plymouth.	a	1939	Spooner, R. verbal communication.

East Coast Marshes.

Wash.	p	1910	Swann, E.L., 1950.
Sheerness.	p	1920	Oliver, F.W., 1920.
Stour.	p	1923	Bingley, J. verbal communication.
Blackwater.	p	1925	Bryce, J., 1931.
Blakeney.	p	1925	Oliver, F.W., in litt. 1927.
Dingwall, Scotland.	p	1937	Braid, J.W. in litt. 1950.
Scolt Head.	a	1939	Chestney, R. verbal communication.
Pegwell Bay.	a	1940	Pettet, A. verbal communication.

West Coast Marshes.

Severn.	p	1914-1923	Roper, I., 1922; Bracher, R., 1933.
Solway.	p	1919	Braid, J.W. in litt., 1950.
Dovey.	p	1920	Yapp, R.H., 1923.
Dee.	a	1930	Pratt, M.C., 1953.

p = planting definitely established.

a = "arrived", no recorded planting.

APPENDIX 3: EXPERIMENTAL MORPHOLOGY - SWARD QUADRAT SAMPLES.

A. Untreated Quadrats: Above Ground Samples.

	Healthy Sward						Pan "die-back"						Channel "die-back"						Dwarfed Sward [y]				
	1955 Sept.	Nov.	1956 Jan.	x Mar.	May	Aug.	1955 Sept.	Nov.	1956 Jan.	x Mar.	May	Aug.	1955 Sept.	Nov.	1956 Jan.	x Mar.	May	Aug.	1955 Sept.	1956 Jan.	x Mar.	May	Aug.
Flower No.	15	4	1	0	0	0	5	4	0	0	0	0	2	0	0	0	0	3	22	0	0	0	23
Live Shoot No	66	56	50	31	47	63	30	70	66	37	71	39	42	23	44	13	54	30	152	110	45	172	264
Live Shoot Wt. (g)	145	146	78	22	8	142	50	113	47	7	25	61	78	45	44	1	11	39	51	10	1	13	62
Live Shoot avg.ht.(mm)	447	437	368	241	126	370	250	221	191	105	160	234	256	271	270	60	137	260	112	88	36	81	140
Live shoot avg.lf. No.	6.7	7.0	6.1	5.3	2.9	5.2	7.3	7.0	5.8	4.7	4.0	5.4	6.5	6.6	5.9	3.0	3.3	4.7	4.6	3.6	2.7	3.6	4.0
Dead Shoot No.	49	41	29	84	55	51	11	4	26	38	45	32	9	3	4	22	23	3	3	73	70	56	40
Dead Shoot Wt. (g)	57	64	43	74	42	56	19	8	17	26	23	20	7	2	2	11	6	1	1	10	6	10	7

x Frost caused the death of many stems in March 1956.

y The dwarfed site was inaccessible in Nov. 1955.

B. Untreated Quadrats: Below Ground Samples.

	Healthy sward		Pan "die-back"		Channel "die-back"		Dwarfed Sward	
	Live(g)	Dead(g)	Live(g)	Dead(g)	Live (g)	Dead (g)	Live (g)	Dead (g)
1955 Sept.	10.9	21.0	4.4	29.9	2.1	16.4	2.7	22.1
Nov.	11.5	13.8	11.6	12.0	6.2	16.1	inaccessible[y]	
1956 Jan.	15.8	9.4	9.9	17.2	0.4	8.6	7.2	13.8
March[x]	2.8	3.1	2.6	14.2	2.4	14.7	8.9	20.8
May	7.6	6.6	1.7	13.8	1.1	15.3	3.2	20.8
Aug.	4.0	1.5	3.7	32.0	2.9	13.9	5.6	44.8

x Frost caused the death of many stems in March 1956.

y The dwarfed site was inaccessible in Nov. 1955.

C. Cut Quadrats: Above Ground Samples.

	Healthy Sward						Pan "die-back"						Channel "die-back"					
	Uncut 1955 Sept.	Cut Nov.	1956 Jan.	x Mar.	May	Aug.	Uncut 1955 Sept.	Cut Nov.	1956 Jan.	x Mar.	May	Aug.	Uncut 1955 Sept.	Cut Nov.	1956 Jan.	x Mar.	May	Aug.
Flower No.	15	0	0	0	0	11	1	0	0	0	0	0	2	0	0	0	0	0
Live Shoot No.	66	44	55	26	116	78	22	5	8	6	19	21	42	9	17	17	37	31
Live Shoot Wt. (g)	145	12	6	1	12	66	58	2	2	1	4	10	78	3	2	1	6	25
Live Shoot Avg.Ht.(mm)	447	65	69	49	101	151	253	96	80	43	120	118	256	76	71	53	122	248
Live Shoot Avg. lf. No.	6.7	3.4	2.7	2.1	1.8	3.9	7.8	4.0	3.0	2.0	3.5	3.8	6.5	4.2	2.7	2.5	3.2	4.6
Dead Shoot No.	49	0	0	0	0	0	8	0	0	0	0	0	9	0	0	0	0	0
Dead Shoot Wt. (g)	57	0	0	0	0	0	16	0	0	0	0	0	7	0	0	0	0	0

x Frost caused the death of many stems in March 1956.

D. Cut Quadrats: Below Ground Samples.

	Healthy Sward		Pan "die-back"		Channel "die-back"	
	Live (g)	Dead (g)	Live (g)	Dead(g)	Live(g)	Dead (g)
Uncut 1955 Sept.	10.9	21.0	2.1	19.5	2.1	16.4
Cut Nov.	8.3	11.3	1.2	26.4	5.2	14.7
1956 January.	3.6	10.8	2.4	11.8	0.1	14.3
x March.	4.9	16.6	0.1	15.6	1.9	18.0
May	7.0	7.8	0.4	16.9	2.9	16.2
August.	5.9	9.0	0.5	27.7	2.4	21.4

x Frost caused the death of many stems in March 1956.

E. Fertilised Quadrats: Above Ground Samples.

	Healthy Sward						Pan "die-back"						Channel "die-back"					
	Unfert. 1955 Sept.	Fert. Nov.	1956 Jan.	x Mar.	May	Aug.	Unfert. 1955 Sept.	Fert. Nov.	1956 Jan.	x Mar.	May	Aug.	Unfert. 1955 Sept.	Fert. Nov.	1956 Jan.	x Mar.	May	Aug.
Flower No.	10	2	3	0	0	0	3	5	0	0	0	1	0	1	0	0	0	0
Live Shoot No.	64	26	33	28	68	34	23	37	48	62	58	55	32	28	43	22	49	30
Live Shoot Wt. (g)	165	58	66	8	15	62	32	60	55	18	22	115	37	54	47	6	15	90
Live Shoot Avg.Ht.(mm)	371	340	354	118	125	301	245	280	240	123	156	268	233	289	276	114	173	376
Live Shoot Avg.lf. No.	6.0	6.0	6.0	4.8	3.0	5.0	7.3	8.6	6.5	4.5	4.0	5.2	6.4	7.5	6.3	4.5	4.0	5.7
Dead Shoot No.	27	35	36	28	28	39	13	16	37	39	36	34	26	14	16	34	33	30
Dead Shoot Wt. (g)	42	89	67	28	39	47	16	17	37	30	25	28	23	25	17	27	23	28

x Frost caused the death of many stems in March 1956.

F. Fertilised Quadrats: Below Ground Samples.

	Healthy Sward		Pan "die-back"		Channel "die-back"	
	Live (g)	Dead (g)	Live (g)	Dead(g)	Live (g)	Dead (g)
Unfert. 1955 Sept.	10.5	18.2	4.6	23.6	3.4	12.1
Fert. November	3.2	17.1	3.9	12.8	0.7	9.8
1956 January.	7.4	31.1	1.1	13.9	3.7	17.7
March[x]	0.9	16.7	5.6	19.0	0.1	4.4
May	2.9	15.8	2.0	23.3	3.1	12.3
August	2.9	13.0	2.1	9.3	4.2	16.3

[x] Frost caused the death of many stems in March 1956.

G. Cut and Fertilised Quadrats: Above Ground Samples.

	Healthy Sward						Pan "die-back"						Channel "die-back"					
	Uncut 1955 Sept.	Cut Nov.	z 1956 Jan.	x Mar.	May	Aug.	Uncut 1955 Sept.	Cut Nov.	1956 Jan.	x Mar.	May	Aug.	Uncut 1955 Sept.	Cut Nov.	1956 Jan.	x Mar.	May	Aug.
Flower No.	10	0	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	2
Live Shoot No.	64	24	9	6	4	0	23	17	5	4	30	6	32	12	28	15	42	35
Live Shoot Wt. (g)	165	9	2	1	1	0	32	14	3	1	14	8	37	4	9	1	8	74
Live Shoot Avg. ht.(mm)	371	78	86	52	65	0	245	136	178	75	166	200	233	90	93	71	117	302
Live Shoot No. Avg. lf.	6.0	3.7	4.3	3.0	2.3	0	7.3	5.7	4.8	2.0	4.4	5.1	6.4	4.3	3.3	2.8	3.5	5.3
Dead Shoot No.	27	0	0	0	0	0	13	0	0	0	0	0	26	0	0	0	0	0
Dead Shoot Wt. (g)	42	0	0	0	0	0	16	0	0	0	0	0	23	0	0	0	0	0

x Frost caused the death of many stems in March 1956.

z Algae began to form as a mat on the cut and fertilised healthy sward in January 1956.

H. Cut and Fertilised Quadrats: Below Ground Samples.

	Healthy Sward		Pan "die-back"		Channel "die-back"	
	Live (g)	Dead (g)	Live (g)	Dead (g)	Live (g)	Dead (g)
Uncut 1955 September	10.5	18.2	4.6	23.6	3.4	12.1
Cut November	7.6	36.1	2.1	20.4	3.0	7.8
1956 z January	4.9	11.2	0.5	12.9	3.1	15.4
March x	0.9	14.2	2.2	18.8	1.2	10.7
May	4.0	18.8	1.5	25.2	1.8	9.4
August	0.0	34.4	2.6	6.4	0.8	5.1

x Frost caused death of many stems in March 1956.

z Algae began to form as a mat on the cut and fertilised healthy sward in January 1956.

I. Pan drained and Pan blocked: Above Ground Samples.

	Incipient pan drained						Incipient pan blocked.					
	1955 Sept.	Nov.	1956 Jan.	x Mar.	May	Aug.	1955 Sept.	Nov.	1956 Jan.	x Mar.	May	Aug.
Flower No.	9	2	0	0	0	0	2	0	0	0	0	0
Live Shoot No.	60	42	19	14	18	53	31	14	17	17	25	11
Live Shoot Wt. (g)	105	49	14	1	4	77	44	27	23	3	17	28
Live Shoot Avg. ht (mm)	214	195	197	83	111	254	229	317	301	104	180	284
Live Shoot Avg. lf. No.	6.0	5.9	5.5	4.0	3.1	4.6	5.3	6.3	5.3	4.6	4.2	5.6
Dead Shoot No.	11	20	12	30	12	34	10	5	7	31	13	8
Dead Shoot Wt. (g)	26	17	9	22	7	25	25	9	9	28	8	9

x Frost caused the death of many stems in March 1956.

J. Pan drained and Pan blocked: Below Ground Samples.

	Incipient pan drained		Incipient Pan blocked	
	Live (g)	Dead (g)	Live (g)	Dead (g)
Untreated 1955 September	3.1	13.6	7.2	18.3
Treated November	3.3	24.0	0.6	13.1
1956 January	0.0	14.2	0.0	7.6
March x	0.3	22.2	1.5	23.3
May	0.6	12.8	1.8	11.5
August	0.4	47.3	1.1	28.2

x Frost caused the death of many stems in March 1956.

APPENDIX 4: MEASUREMENT OF SOIL SULPHIDES.

I. METHOD.

A. Principle.

As soon as possible (within 3 hours) after collection the soil was suspended, or the soil solution run into, air free water in an efficient annular bubbler. The bubbler had nitrogen blown through it from a cylinder. The nitrogen stream saturated the soil suspension or solution and swept out hydrogen sulphide from the dissolved soil sulphides. All sulphide was swept from soil solutions by this method, but in the case of *Spartina* soils there are considerable "reservoirs" of undissolved ferrous sulphide which replenish the sulphide in solution, until a new equilibrium is reached. The amount of sulphide obtained from soils in this way may be termed "readily available sulphide", and seems to be a definite quantity for any given soil.

The gas stream from the bubbler (nitrogen plus hydrogen sulphide) was run through a Pettenkopfer tube containing dilute caustic soda solution to absorb the hydrogen sulphide. A tube of colorimetric reagent, beyond the absorbing solution, checked that no sulphide escaped absorption.

Aliquots were taken from time to time, and for this the gas stream was stopped. These were used for colorimetric sulphide determination against a bismuth-acetic acid reagent (Snell, F.D., and Snell, C.T., 1949). When a constant reading was reached, this was taken as the total readily available sulphide value.

On the soil suspension in the bubbler, a determination of the remaining sulphides could also be made by running in hydrochloric acid, in the closed apparatus, and sampling the caustic soda absorbing solution until a second end point was reached. This was taken as the remaining sulphide value plus the readily available sulphide already determined. The remaining sulphide value was obtained by subtraction. Citric acid trials showed that even this remaining sulphide was probably available to plants, though less so than the "readily available sulphide".

B. Soil Sampling.

Soils were collected by a modification of the Warcup anaerobic bacteriological technique, due to Miss Clitheroe (1955). Specimen tubes 4" x 1" were supplied with glass tubes reaching to the bottom, and bent over the rim of the specimen tube to extend backwards a short distance. When these were pushed into wet soil the glass tubes released the air otherwise trapped in the bottom of the specimen tube. After sampling, the glass tubes were withdrawn, and the specimen tubes stoppered with slit corks, leaving no air between the sample and the cork.

C. The Bubbler.

1. Construction.

The bubbler (Fig. 31A) was made from a length of ¾" outside diameter glass tubing (A). The lower end (A_1) was almost sealed, leaving an orifice of ¼" diameter. The upper end (A_2), 7" above A_1, was drawn out and bent to a right angle. This unit (A) was set in a rubber bung (B) which also held a short outlet tube (C).

The bung (B) with its tubes (A) and (C) was fitted into a boiling tube 7" x 1½" (D). The specimen tubes used in sampling fitted inside the boiling tube. This is shown with a specimen tube in place (E).

2. Method of use.

a) Preparation and "Readily available sulphide" determination.

(For soils):

The boiling tube was filled with boiled (air free) distilled water. The specimen tube was uncorked and dropped into the boiling tube, immersed in the water so that no air bubbles remained between the specimen tube and the boiling tube. A glass rod was pushed through the soil in the specimen tube and used to punch out the

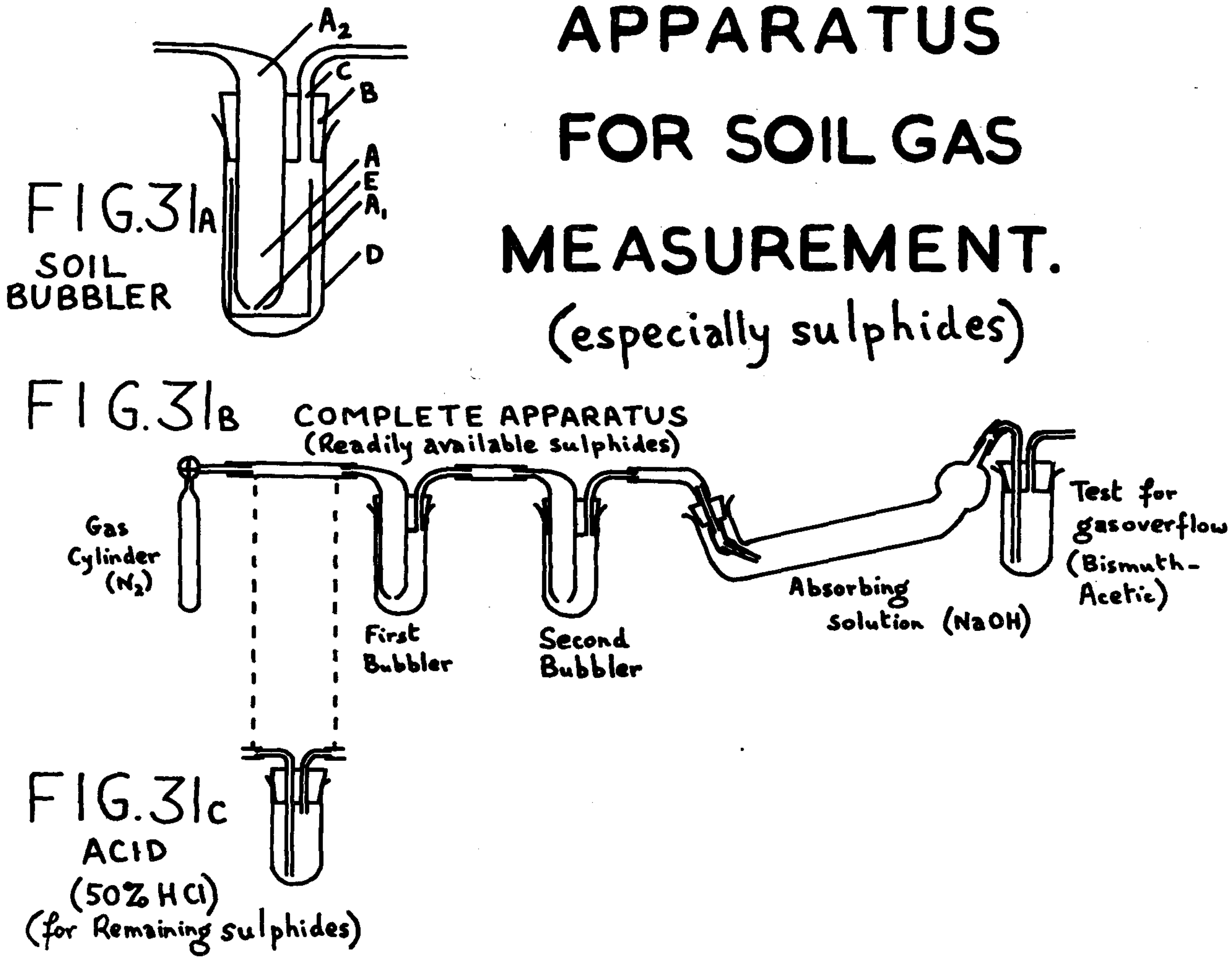


FIG. 31. Soil gas analysis technique.

glass bottom of the tube. When this was done, the wall of the specimen tube was removed in one piece, leaving the soil under water, only the broken bottom of the specimen tube being left (if any more is left the efficiency of bubbling through the suspension is impaired).

(For soil solutions):

The solution was run into the boiling tube of air free distilled water, with as little disturbance as possible.

(For soils and solutions):

The boiling tube, with the soil suspended in it, or the solution in it, has the bubbling tube lowered into it, with a slow nitrogen stream flushing through. This keeps the orifice clear as the bubbler goes into place, and flushes out any air which may be left. Some soil suspension is displaced by the bubbler, and this goes up the outlet tube, and into a similar bubbler in series with the first. The second bubbler has a slightly wider orifice than the first to prevent blockage by soil suspension. As soon as the bung is home it is bound in position, the nitrogen running all the time. The remainder of the apparatus, previously nitrogen flushed, has to be attached to the second bubbler from the beginning, so that the nitrogen stream can be run continuously. Stopping the nitrogen stream usually leads to blockages. The apparatus as it is in use is shown in Fig. 31B.

The bubbling was continued until no more hydrogen sulphide was released (detected by momentarily stopping the nitrogen stream and taking aliquots of the absorbing solution). This gave the sulphide content of solutions, and the "readily available sulphide" values for soils.

b) Remaining sulphide determination.

For remaining sulphide determinations on soils, after the previous procedure, the nitrogen stream was disconnected and the entry tube of the first bubbler clipped. A test tube of 50% hydrochloric acid was then added to the apparatus, between the nitrogen cylinder and the first bubbler (Fig. 31C). This was flushed free of air, and connected to the bubbler. By inverting this tube, acid was run into the soil suspension, some of which overflowed into the second tube. Gradual addition and bubbling were carried out, with shaking, until the suspensions of soil changed colour from black to grey, and there was no rise in the sulphide detected between one aliquot and the next. Bubbling to the end point usually took ½ - 1 hour.

D. Absorption and determination of hydrogen sulphide.

1. "Readily available sulphide".

The hydrogen sulphide swept through the apparatus was removed from the nitrogen stream by absorbing in caustic soda solution in a Pettenkopfer apparatus. For readily available sulphide determination, a minimum volume of 6% NaOH was used (20 ml.). Aliquots of 5 ml. were taken, and added to an equal volume of the bismuth-acetic acid reagent (Snell, F.D. & Snell, C.T., 1949) in a colorimeter tube, and nitrogen was bubbled through for 30 seconds, then the suspension of brown bismuth sulphide was allowed to stand for 5 minutes, and finally the transmittance read with an "EEL" colorimeter, using filter 621 violet.

2. "Remaining sulphides".

For the determination of the more concentrated remaining soil sulphides, the 6% NaOH solution in the Pettenkopfer tube was made up to 60 ml., and 0·1 ml. aliquots taken.

E. Calibration, limits and accuracy of the method.

The colorimetric reagent was standardized by reading the transmittance of solutions made with known sulphide strengths, titrated iodimetrically (Vogel, A.I., 1948).

The lowest quantity of sulphide detectable by the method was on the order of 1×10^{-5}g.S^{--}/100g. soil. The greatest quantity was on the order of 1×10^{-1}g.S^{--}/100g. soil. The upper limit was set by the strength of solution causing flocculation of the precipitated sulphide. Transmittance could only be read on the colloidal suspensions. Use of a larger volume Pettenkopfer type absorbing tube would increase the upper limit slightly, but the range of values covered is probably that most useful in soils. An accuracy within 5% of the mean can be obtained on triplicate soil samples.

II. OTHER USES OF THE METHOD.

The method was successfully used for CO_2 and carbonate determinations, with baryta titrations. It may be of use for other gases, except perhaps oxygen where the risk of contamination from the atmosphere is especially great.

APPENDIX 5: NOTES FOR THE BIOLOGICAL FLORA.

(Figure numbers refer to thesis diagrams).

Biological Flora of the British Isles.

Spartina townsendii H. & J. Groves.

Spartina townsendii was first collected in, or just before, the year 1870 at Hythe in Southampton Water (Sutherland & Eastwood, 1916) but was not described as a new species until 1881 (Groves, H. & J., 1881; 1882). Although known in Norfolk as cord grass, it is more commonly called rice grass in many parts of this country, where it is supposed it originated from the cargo of a wrecked rice ship, and also in Europe. The species was suggested by Stapf (1908) to be a naturally produced hybrid of *S. maritima* and *S. alterniflora*. A similar explanation had been advanced for a French counterpart species (Foucaud, 1897). Some cytological evidence for the hypothesis was advanced by Huskins (1930) who examined fertile material ($2n = 126$), *S. maritima* ($2n = 56$), and American *S. alterniflora* ($2n = 70$) no British material being found. The existence of male sterile *S. townsendii* ($2n = 63$) went unnoticed until recently (Benoit, P., Hubbard, C.E., Chater, E.H., and Thomas, P.T.). Examination of herbarium material has shown that before 1910 the plants were apparently exclusively of the male sterile type, providing further evidence for the Stapf hypothesis. The male sterile form is still found today on the south coast, especially near Hythe, and locally elsewhere in the considerable swards of the fertile form. A description of the male sterile form follows the account of the more common fertile form.

Perennial with rhizomes bearing scale leaves and irradiating to form tufts 30 - 40 cm. from parent stock. Tufts consisting of a cluster of aerial shoots, strong roots, and new rhizomes, increasing annually by growth at the periphery. Aerial shoots normally grow until a flowering head is produced. Overwintering shoots produce flowering heads in the following summer.

Culm enclosed by from five to thirteen sheaths, the longest some 10 - 15 cm. long; strongly articulated to the blades. Ligule of hairs (about 2.5 mm. long) at the articulation. Blade broadest at base, 7 - 15 mm. wide, with strong shoulders projecting on either side of the stem. Blade usually 15 - 45 cm. long, smooth, dark and shiny on the inferior-external surface, with a faint transverse ridge near the tip. Superior-internal surface greyish green on account of papillae on the ridged surface (Jacquet, 1949). Leaf usually flat or slightly inrolled, standing at an angle to the stem.

Inflorescence, 10- 25 - (35) cm. long, a close raceme formed by early division of the spikes. The 2 - 4 - 5 - (12) spikes (7) - 10 - 23 cm. long, suberect or slightly spreading, rigid, overtopping the leaves, bearing narrowly oblong spikelets, 16 - 20 mm. long, 3 mm. wide, flattened and closely overlapping, borne on short pedicels attached at an angle into hollows on two sides of the rachis which is smooth, triangular, flexuose and produced beyond the spikelets by up to 4 cm. Glumes unequal; superior delicately pubescent, with a keel ciliate to the hyaline tip; the base with a hyaline border; 14 - 19 mm. long, 3 - 6 nerved; almost encasing the palea; narrow and lanceolate. Inferior glume delicately pubescent with short, straight hairs and a ciliate keel ending abruptly below the short hyaline tip (Stapf, 1908); 10 - 13 mm. long, 3 mm. wide, 1 - 3 - 6 nerved; shorter than lemma; narrowly lanceolate to almost subulate. Lemma lanceolate-oblong shorter than palea, with hyaline margin, 1 - 3 nerved, minutely hairy upwards, a little shorter than superior glume, 2 nerved, acute or subacute, glabrous. Florets one per spikelet, rarely two. Lodicules absent. Protogynous, styles feathery, 2 : 10 mm. or more; stamens 3; anthers 8 - 11 mm. exerted at maturity on long filaments. Pollen yellowish, shiny spherical fertile grains. Fruit a caryopsis; viviparous.

In addition to the male sterile form, both genetic and environmental forms occur. A dwarf brown mutant

(2n = 122) matures at 25 cm. height in the Dovey estuary. It has deep purple-brown leaves, and grows in tussocks beside those of the (2n = 126) form which in similar positions attains full height. A variegated mutant (2n = 98) also grows in the Dovey estuary, though less commonly. This attains full height for S. townsendii, only differing from the type in having yellow stripes down the leaves (Thomas, P.T., & Chater, E.H.).

In strongly wave eroded conditions and on shingle a dwarf environmental form is found, growing to 25 cm. height, and slightly pigmented. This form is easily distinguished from the dwarf brown mutant by the general and obvious cause of its reduction, and its growth to normal proportions when cultured.

Native along the south coast of England and in Northern France. Introduced on the east and west coasts of Great Britain, and in many places throughout the world.

I. Geographical and altitudinal distribution.

Distribution and abundance in the British Isles.

Along the south coast on all salt marshes from Poole in the west to Pagham in the east, including the Isle of Wight, there have been Spartina stands for 40 - 70 years, and the plant is well established, (Stapf, 1908; Oliver, 1920; 1925).

On the east and west coasts south of a line from the

Humber to the Mersey, marshes with Spartina occur in all littoral vice-counties except five (Fig. 5). None of these stands is more than 40 years old, and they were mainly started from plantings. They vary greatly in vigour and density, according to the suitability of the planted site (Uren, E.).

Spartina has also been reported from Ireland and Scotland, in four areas where it was evidently planted (Cummins, 1930; Doyle, 1934; Braid).

Extra British Distribution.

Planted at Fanø, Denmark (Jørgensen, 1931), West Schleswig Holstein (Konig, 1949) and Holland (Vries, 1949; Jørgensen, 1931), and it has spread through Northern France in estuaries and salt marshes as far south and east as the Baie de la Fresnaye near St. Malo (Corillion, 1951). Also successfully planted in Australia and New Zealand, though much New Zealand material is sterile (Hubbard, C.E.). Attempts to establish it in Honolulu, Bengal, Jamaica and Hong Kong have met with no recorded success. As a result of planting trials, the geographical limits were described as the 20th degree north and south to the 52nd degree south and the 56th degree north, (Martyn and Bryce, 1941). It is now known to succeed up to the 58th degree north, at Dingwall, Scotland.

II. Habitat.

(a) Climatic and topographical limitations.

The limits of distribution may be set by the summer temperature, the northern limit of successful plantings corresponding to a July isotherm of 56°F., and the southern to 50°F.

The species is somewhat dependent on topographical features, growing inside the lees of shingle banks, harbours, estuaries, and other places rather than facing the open sea where the substrate is rarely stable or adequately protected from wave action.

The upper limit of the species lies near M.H.W.O.S.T., the lower limit being primarily controlled by time of immersion (usually ca. 6 hrs.), depending on the slope of the shore and the tidal range, and probably also depending on drainage, salinity, and substrate. The plant occupies the zone 5 - 8 ft. above L.W. at Lymington, where the tidal range is 8 ft., but the zone 31 - 37 ft. above L.W. at Stert in the Bristol Channel (range 37 ft.).

(b) Substratum.

The parent material of the Spartina marsh may be any of the common salt marsh substrates, from almost pure sand, as on the west coast, to sandy clay as on the east coast, and fine wet silt and clay on the south coast.

Locally on muddy shingle (Stone Point, Essex Stour) and pure sand (Sandbanks, Poole) only feeble growth exists. The layering of materials differs, from sand and clay supporting strong growth (Norfolk), with good drainage, to almost unrelieved wet silt and clay for the top 2 metres, supporting poor growth (South coast), and with poor drainage.

Spartina accretes a characteristic fine silt and clay from the river washings it receives in most habitats, and the finest, softest, wettest silts and clays accumulated in this way may give rise to sward degeneration - "die-back" - after a period of colonization (Goodman, P.J.). Physical and chemical analyses produced the following results for the surface layers of soil at Lymington, (Table I).

TABLE I.

Physical and chemical analyses of *Spartina* soil at Lymington.

	Strong growth	"Die-back"
Physical data.		
Water Content	26 - 31%	76 - 84%
Organic Matter (Ignition)	5%	24 - 30%
pH (pH Meter)	6.8	6.6
Redox potential (E_7)	170 - 240 mV	negative
Chemical data.		
Total Nitrogen	0.003%	0.10 - 0.13%
Available Phosphorus	0.006%	0.005 - 0.006%
Potassium	0.0004%	0.0004 - 0.0006%
Calcium	0.05%	0.05 - 0.08%
Sodium chloride	1.5%	1.8 - 2.6%
Carbonate & Bicarbonate	0.09 - 0.11g.%	0.04 - 0.07 g.%
Sulphide	0.00001 g.%	0.036 - 0.075 g.%

The water contents of the soils varied by less than 10% during the period that the plant was uncovered by the tide. Mechanical analyses proved difficult to perform, owing to the deflocculation of the soil particles. No success was achieved with sedimentation methods, but results were obtained by using sieves, washing through with sea water. Typical analyses of Lymington soils are shown in Fig. 27.

In the estuarine muds, especially those of the south coast, are found Corophium grossipes (Crawford, 1937), Nereids, and Carcinas moenas (Austwick, 1950).

III. Communities.

Spartina normally grows in single species stand at the lowest level of the salt marsh. Enteromorpha spp. may be found in association with it on the south coast. Locally, Salicornia spp. occur as co-dominants (e.g., Christchurch) as also may Halimione portulacoides (e.g., Hythe).

Puccinellia maritima is co-dominant on the west coast marshes, though generally occupying a higher level. Chapman (1941) regards the Spartineto-Halimionetum and the Puccinellieto-Spartinetum as ecotones. Salicornia, Puccinellia and Halimione may possibly require slightly sandy conditions. S. townsendii is also found occasionally in Aster dominated marsh (Blakeney) and in Limonium dominated marsh (Newtown, I.O.W.), and may even occur subordinate to Juncus maritimus, Scirpus maritimus, and Phragmites communis, though it seldom, if ever, replaces these, and competition with them occurs only where fresh water enters the marsh.

IV. Response to biotic factors.

Sheep grazing occurs on the sandy west coast marshes, and under heavy grazing pressure it seems that replacement of Spartina by Puccinellia can occur. Spartineta bordering the New Forest are damaged by pony grazing, wet hoof-prints being left uncolonized. The drier ridges of this zone, 10 - 100 yds. wide, being colonized by sparse Puccinellia maritima and Salicornia spp.

Cutting of the sward causes a flush of new growth in the following year if no blanket of algae has settled in the meantime. Algal blankets check new growth. No significant effects followed application of fertilizer to the sward.

V. (a) Gregariousness.

Grows in tussocks centred on the seedling or fragment which initiated them, by peripheral rhizome growth. Observations by Mrs. Caldwell at Blakeney and elsewhere have shown that thinning of the centre occurs where flowering shoots are not immediately replaced, and later up to 8 rings of thin growth are seen, though usually fusion of neighbouring tussocks occurs by this time, and the pattern is lost. Considerable swards are produced by tussock fusion.

(b) Performance in various habitats.

Tussock increase of ca. 1 metre in diameter yearly has been reported from the Dovey (Chater, E.H. & Jones, H.). At Blakeney shoot density in tussocks varies from 3 - 50 shoots per 300 sq. cm. (Caldwell, P-A.). At Lymington sward stem density varies from 13/1,000 sq. cm. on "die-back" edges to 264/1,000 sq. cm. on wave eroded fronts. Healthy sward normally has about 50/1,000 sq. cm. Seedling densities up to 8/1,000 sq. cm. have been recorded in sward, though the seedlings seldom survive. Dense seedling growth may occur on uncolonized mud, seedlings almost touching for areas of 1,000 sq. cm. or more. Flowering density varies from zero to 23/1,000 sq. cm., representing up to one flowering head in four.

Reduced growth is found locally on shingle, sand, and in eroded sites, at Stone Point, Essex; Sandbanks, Poole; and Eastlake, Lymington. The tallest growth, up to one metre or more, is found on fine sandy silts, with a little clay, as in the Dovey, and locally at Sandbanks, Poole. In the very soft wet silts of the south coast, and particularly at Lymington, growth failure - "die-back" - occurs, the plants yellowing, with weak roots and rhizomes, which eventually develop a soft rot. Few tillers are produced and the plants become moribund, the proportion of young parts becoming less until the plants fail altogether (Goodman, P.J.).

Limited growth occurs in garden soil but <u>Spartina</u> is successfully cultured in pots of sand well watered with fresh water and occasionally with Knop's solution with a trace of manganese added.

(c) Effect of frost and drought.

The plant survives periods of cold in its first winter as a "viviparous" embryo. In the exceptional winter of 1955 - 1956 on the south coast the majority of the overwintering stems were killed by frost and drought effects after freezing of the salt marshes down to M.L.W.O.S.T.

High humidity is needed for successful germination, but mature plants are less sensitive to drought. Frost and drying cause similar symptoms, leaf rolling and erection, causing damage at the pulvinar articulation.

VI. (a) Morphology.

Spartina plants consist of stems bearing opposite and alternate leaves with axillary buds in groups of three, the centremost giving rise to a stem, the outer two to roots.

1. Tillers and rhizomes,

Vegetative reproduction begins from central axillary buds on the winter rosette stems. All stem buds appear similar, but the upper, and if the stem is slanting, the backwardly and upwardly directed buds generally give rise to tillers, while the forwardly directed buds give rise to rhizomes.

Tiller buds grow vertically, at first inside the leaf sheath (intravaginally), producing a new green leaved stem. Rhizome buds grow extravaginally and horizontally underground, with white awl-like leaves becoming scales behind the apex. Rhizomes are strongest near the base of the stock, and forwards, the system advancing. Undamaged rhizomes seldom branch. Axillary buds replace damaged apices. Pioneer rhizomes usually borne at or near the first internodes bearing foliage leaves. Rhizomes at a depth of up to 10 cm., for up to 40 cm. distance, when they turn up with short internodes bearing foliage leaves, initiating new tufts.

2. Roots.

Adventitious roots form from outer axillary buds of winter rosettes: Simple roots on the horizontal rhizome nodes. Rosette roots dimorphic - thick, long, white, unbranched, nearly hairless and descending to a metre or more; and slender, white, black (reduced iron) or brown (oxidized iron) stained, with laterals of the third order, spreading in all directions, in upper 10 cm. of substrate. The former are probably anchoring roots, the latter probably primarily absorptive. Transitional types up to 40 cm. long and slightly hairy. More holding roots seem to form in wet conditions. In drier and eroding conditions absorbing roots are especially noticeable. Aeration may control root development.

(b) Mycorrhiza.

None recorded and none observed in preliminary investigations.

(c) Perennation; reproduction.

Rhizome hemicryptophyte overwintering as rosettes of short internodes and clustered leaves, in whose axils buds are borne. These give rise to tillers and rhizomes whose apices turn upwards in spring. In "die-back" fewer buds form, and many die. Rhizomes effect forward spread of the plant, consolidation following by tillering.

Rhizome tips usually remain underground during the winter, though in poor conditions they may grow long and feeble and remain below ground for up to a year. Upwardly growing shoots normally flower in their first year, though late tillers may overwinter and flower in a second year. After flowering, the cluster and its associated roots die.

Flowering in the field occurs in two year old plants, but in long days of temperate greenhouse culture seedlings flower in one year. Cuttings flower after one year in the field, or after six months in the greenhouse. Seed is set every year, though in varying quantity. Reproduction occurs by fragments and seeds. Successful planting occurs from cuttings (Carey & Oliver, 1918).

(d) Chromosome number.

Male sterile (2n = 63) (Thomas, P.T.); Fertile (2n = 126) (Huskins, 1930); Dwarf brown (2n = 122) (Thomas, P.T.); Variegated (2n = 98) (Thomas, P.T.).

(e) Physiological data.

Leaf structure xeromorphic, with strong papillate ribs on the superior-internal surface. Leaves roll when dry, by motor cells, and are erected at the pulvinar articulation (Jacquet, 1949; Sutherland & Eastwood, 1916). Hydathodes among leaf cells reported capable of secreting salt solution (Skelding & Winterbotham, 1939). Seedlings do not germinate in sea water, but germination percentage rises as salinity falls. Salinity of plants is apparently unrelated to osmotic pressure (Jacquet, 1949).

Plant analyses showed that sugars were lowest in spring. Rhizomes contained most sugar in winter, while leaves contained most in summer (Jacquet, 1949). Starch absent in French material, (Jacquet, 1949), but present in English material (Goodman, P.J.). Ascorbic acid content reported to vary with time of immersion (Jacquet, 1949).

Air spaces form in the plant by breakdown of cortical and medullary cells, and are separated at nodes by diaphragms with a high resistance to gas flow. High rhizome CO_2 and alcohol contents may develop in waterlogged conditions (Goodman, P.J.).

VII. Phenology.

Quadrat studies at Lymington showed a marked seasonal rhythm of growth. In March plants had rosette form with few leaves and scarcely developed tiller buds. Dead culms persisted from autumn flowering. Upturning rhizomes left dead parts below while forming rosettes at the soil surface. Spring growth began in April or May. Flowering began May - June, becoming vigorous in June, and continuing so through September. In summer months there was little live material below ground.

In November many tall shoots flowered and died, and stem growth slowed. Axillary bud growth began, and rhizomes developed below ground, especially in strong sward. The first fruits ripened in November, falling off in winter storms, complete in their flowering glumes. Germination began on the drift line in November, but was greatest in spring after frosts. Flowering finally finished in February or earlier if frosty.

VIII. (a) Floral biology.

Anemophilous. Protogynous. Stigmas emerging 7 days after spike emergence and pollen mother cell formation. Stigmas continue to grow for 7 days unless pollinated (Curtiss, 1937). Stamens emerge 5 days later, pushing stigmas aside. Anthesis first in mid-spike, in the morning, anthers splitting as they emerge, freeing themselves in about 2 hours. Filaments elongate rapidly for 2 further hours, then wither (Goodman, P.J.). No recorded cases of self-fertilization, cleistogamy, or apomixis. Caryopses viviparous, the embryos having no resting period (van Schreven, 1952).

(b) Hybrids.

No evidence for the existence of hybrids.

(c) Seed production and dispersal.

In 12 collections from Britain and Holland in 9 different years (Curtiss, 1937; Nelson & Munro, 1934; Goodman, P.J.), the seed set averaged 58%, ranging from 18% (February, 1955) to 92% (March, 1954), both at Lymington. The 2 - 4 - 5 - (12) spikes bear 15 - 50 spikelets per spike. 1, rarely 2, flowers per spikelet. Spikes develop evenly, lack of seed probably being due to failure of ovary development or after pollination. Terminal spikelets usually sterile. Good seed has a hard endosperm and green scutellum. Bad seed is brown and soft. Seed set is apparently unrelated to locality or time of year, and is probably climatically controlled.

Entire spikelets are tide borne, floating by air trapped in the glumes. Becoming waterlogged, they remain on the drift line, especially after November tides. They are carried long distances by currents, shipping, and probably on the feet of wading birds.

(d) Viability of seeds; germination.

Germination tests have shown that 7 weeks storage at room temperature makes seed inviable (Nelson & Munro, 1934), but storage over water keeps seed viable for 4 months at room temperature, for over 12 months at 5°C. Low temperature storage produces clean, easily germinating seed (Goodman, P.J.).

Seedlings develop quickly at 25°C. in high humidity. Salt water inhibits germination. 95% seedlings which received no cold treatment died after 15 weeks, and Jacquet (1949) reports a similar effect in seedlings which have received no salt. Seedlings apparently need either a period of cold or of immersion in salt water to develop successfully.

(e) Seedling morphology.

The coleoptile first emerges from between the flowering glumes, splitting the caryopsis coat. Next the coleorhiza appears from the other end of the mesocotyl. The first leaf penetrates the coleoptile; the seminal root pushes through the coleorhiza, and the seedling is established. New leaves emerge with distinct sheaths and laminae. The plastochrone is 4 weeks outdoors, and $2\frac{1}{2}$ weeks in the temperate greenhouse, in June. Adventitious roots appear after 8 - 10 weeks, and the mesocotyl and seminal radicle wither after about 20 weeks. Whereas leaf production rate increases with temperature, stem growth rate increases with daylength. This may be due to the greater light quantity received, or to a true photoperiodic effect.

The first stem buds become visible to the naked eye after about 15 - 20 weeks growth, and these are usually tiller buds, not from the lowest axils (Fig. 14). Later, the very lowest axils give rise to rhizomes, generally after 25 weeks. More tillers and rhizomes form in short days than in long days.

(f) Effective reproduction.

Seeds carry the plant to great distances in tidal currents. Once established, the swards are often extended by fragments breaking away under tidal action, and after freezing of the marshes. Such fragments can be detected by examination of rhizome systems of young tussocks. In full sward reproduction from seedlings is unimportant, the sward being maintained by rhizoming and tillering.

IX. (a) Animal feeders and parasites.

Tarsonemus pallidus Banks was found by Dr. A.M.Massey on blackened leaf tips collected at Keyhaven by Austwick (1950). Euscelis obsoletus Kb. was found associated with leaf spots on Spartina at Lymington, and identified by Dr. W.E. China. Sipha littoralis Walker was found on yellowed leaves by Mr. F. Laing (Austwick, 1950).

(b) Plant parasites.

Preliminary investigations by Austwick (1950) and (Goodman, P.J.) revealed a number of saprophytes of Spartina, including Halophiobolus medusa (Ell. & Ev.) Linder. Most were on the dead culms, but it seems unlikely that any were parasitic. ?Ligniera junci (Schw.) Maire & Tyson, was present in living roots.

(c) Diseases.

"Die-back" of the sward results from soil deterioration, without associated pathogen attack. It is confined to estuaries where the finest wet silts accrete, and is associated with highly anaerobic soil conditions. These conditions cause a soft rot of roots and rhizomes, and a reduction in the production and survival of tiller buds. Locally on the south coast "die-back" has caused considerable damage to Spartina swards (e.g., at Lymington).

X. History.

From Hythe the grass spread along the coast to east and west. By 1915 it was established in every estuary and salt marsh from Chichester to Poole (Stapf, 1908; Oliver, 1920 and 1925). Outside this area, the grass was largely planted from 1910 onwards (Goodman, P.J.), often for private reclamation work (Oliver, 1920; Roper, 1922; Bryce, 1931; Yapp, 1923). It has extended from these plantings to cover at least 30,000 acres of England, Scotland, Wales and Ireland, and has spread and been planted in Europe, to stabilize silty areas (Jørgensen, 1934). It has been found to have fair grazing value (Knowles, 1929). The anticipated nuisance value of colonization (Oliver, 1920) was not realised, and recession by "die-back" is causing only local problems (Goodman, P.J.). Probably most suitable substrates are already colonized, but careful planting and management in new areas may yet obtain useful results in land conservation and reclamation from the sea.

Appendix.

Male sterile form of Spartina townsendii. (2n = 63).

More slender and more rigid, with narrower leaves and shorter ligules than fertile plant. Further differing in spikelet dimensions, 14 - 16 mm. long, and 2 mm. wide. Anthers 5 - 7 mm. long, yellowish, incompletely exerted or exerted on short filaments, and not opening. Pollen smaller than fertile pollen, irregular, transparent and imperfect. Styles smaller. Corresponds with type specimen (Herb., Kew).

Form persists near Hythe and in fertile swards along south coast. Apparently introduced accidentally to Barmouth, Wales, and in New Zealand.

Partial anthesis in June, continuing until August, and probably later. Spread must be entirely vegetative, assisted by accidental planting, with fertile material. Otherwise apparently has similar properties and requirements to the fertile form. Nothing further known than this fragmentary information of (Benoit, Hubbard, and Goodman, P.J.).

References.

Austwick, P.K.C. (1950). Report of investigations into the cause of dying out of Spartina townsendii in Lymington Harbour. (Abstracted by permission of Sir E. Salisbury and Mr. Austwick). Report to the Director, Royal Botanic Gardens, Kew.

Bryce, J. (1931). Further notes on economic possibilities of Rice Grass. J. Minist. Agric. 38, 801-805.

Carey, A.E. & Oliver, F.W. (1918). Tidal Lands. London: Blackie.

Chapman, V.J. (1941). Studies in Salt Marsh Ecology VIII. J. Ecol. 29, 69-82.

Corillion, R. (1951). Extension récente du Spartina townsendii Groves en Bretagne. Monde des Plantes 274 2.

Crawford, G. (1937). A review of the amphipod genus Corophium with notes on the British species. J.mar.biol.Ass. U.K: N.S. 22, 589-630.

Cummins, H.A. (1930). Experiments on the establishment of Rice Grass in the Lee. Econ.Proc.R.Dublin Soc. 2, 419-430.

Curtiss, W. (1937). The floral morphology of S. townsendii. New Phytol. 36, 26-32.

Doyle, J. (1934). Spartina townsendii at North Bull, Dublin. Irish Nat. Journal, 5, 158.

Foucaud, J. (1897). Un Spartina inédit. Ac. de la Rochelle, Ann. Sc. Nat. 32, 220-222.

Goodman, P.J. (1957). An investigation of "die-back" in S. townsendii. Ph.D. Thesis, University of Southampton.

Groves, H. & J. (1881). On Spartina townsendii Groves. Rep. bot. (Soc) Exch. Cl. Manchr. 8, 37.

Groves, H. & J. (1882). Spartina townsendii Groves. J. Bot., Lond. 11, 1-2.

Huskins, C. (1930). The origin of S. townsendii. Genetica 12, 531-539.

Jacquet, J. (1949). Recherches écologiques sur le littoral de la Manche. pp. 374. Paris: Lechevalier.

Jørgensen, C.A. (1931). Om et nyt Marsk-Graes og muligheden for dets anvendelse I vore Marskegne NaturensVerden 1931.

Jørgensen, C.A. (1934). Plantnings forsøg Med Spartina townsendii I den Danske Vesterhavs marsh. Bot. Tidsskr. 42, 420-440.

Knowles, F. (1929). The economic possibilities of Rice Grass. J. Ministr.Agric. 35, 934-939.

Konig, D. (1949). Die Ausbreitung des Grases Spartina townsendii Schleswigholsteinischen Westküste. Forsch. u. Fortschr. 25, 84-87.

Martyn, E. & Bryce, J. (1941). The economic exploitation of Rice Grass. Emp.J.exp.Agric. 9, 167-170.

Nelson, A. & Munro, J. (1934). The germination of Spartina townsendii. Notes of Roy.Bot.Garden, Edinburgh, 18, 173-180.

Oliver, F.W. (1920). Spartina problems. Ann.appl. Biol. 7, 25-39.

Oliver, F.W. (1925). Spartina townsendii, its mode of establishment, economic uses and taxonomic status. J.Ecol. 13, 74-91.

Roper, I. (1922). Spartina and coast erosion. Kew Bull. 5, 351-352.

Skelding, A.D. & Winterbotham, J. (1939). The structure and development of the hydathodes of S. townsendii. New Phytol. 38, 69-79.

Stapf, O. (1908). Spartina townsendii. Gardeners' Chronicle 1,099, 33-35.

Sutherland, G.H. & Eastwood, A. (1916). The Physiological

Anatomy of Spartina townsendii.
Ann.Bot., Lond. 30, 333-351.

van Schreven, A.C. (1952). The fruit of S. townsendii.
Koninkl. Nederl. Akad. Wetensch. 55, 150-162.

Vries, V. de. (1949). Zilte plantengemeenschappen
op Vlieland, Holland.
Levende Natuur 52, 143-152.

Yapp, R.H. (1923). S. townsendii on the Dovey salt
marshes. J. Ecol. 11, 102.