BACTERIAL RHIZOSPHERE INTERACTIONS IN HYDROPONICALLY GROWN WHEAT

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ABSTRACT: Hydroponic culture will likely be used for crop production as part of a Controlled Ecological Life Support System during long term space travel or habitation. To better understand the bacterial component of such a system, the interaction between root-attached and suspended bacteria was studied over the life cycle of hydroponically grown wheat. Total numbers based on viable counts reached an equilibrium in the attached (10^8- 10^9/g dry wt root) and unattached (10^5/mL) communities. Total counts of suspended bacteria were significantly greater ($p=0.05$) on R2A medium, which is designed to promote recovery of slow-growing or stressed bacteria, when compared to nutrient agar or a simulated root exudate medium. Media had no significant effect on total counts of attached bacteria. Data indicate that the rhizoplane microflora represent the numerically and, probably, metabolically dominant component of the microbial load in hydroponic systems. Over 1000 randomly selected bacterial isolates from attached and suspended communities were tested for specific physiological functions. The two communities were not different from one another but did show significant differences with time ($p=0.05$). This study suggests that suspended bacteria result from root sloughing and that nutrient solutions will not support a large indigenous microbial community.

PURPOSE

- Expand the knowledge of microbial dynamics in hydroponic systems

- Determine the relationship between bacteria attached to the root (rhizoplane) and suspended in the hydroponic solution.

- Develop more effective monitoring techniques.

OBJECTIVES

- Enumerate bacterial populations over the life cycle of a hydroponically-grown plant (wheat)

    - Rhizoplane community
    - Suspended community

- Characterize the microbial community composition based on ecologically relevant functional tests

METHODS FOR ENUMERATION & CHARACTERIZATION
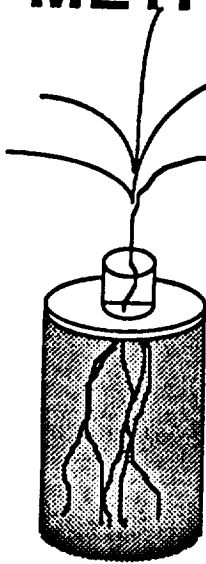
- 36 REPLICATE PLANTS
- STERILE 6 DAY WHEAT SEEDLINGS
- STERILE HOAGLAND'S SOLUTION
- AERATED
- INDIGENOUS MICROFLORA
  FROM 10 mL of WHEAT NFT
  HYDROPONIC SOLUTION

- 4 REPLICATES AT 9 DATES
  SAMPLED DURING GROWTH CYCLE
- DILUTION SERIES SPREAD PLATED
  ONTO THE FOLLOWING MEDIA

NUTRIENT AGAR

EPA R2A

REM
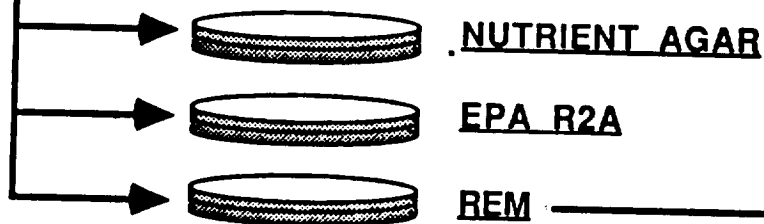
A note on the media used:
- Nutrient agar (Difco)
  - High concentration, protein based
  - Commonly used for rhizosphere microbiology
- EPA R2A (Difco)
  - Low concentration, carbohydrates and proteins
  - Designed for recovery of injured or stressed bacteria from potable water
- REM (designed for this study)
  - arabinose, rhamnose, fructose, xylose, glucose, casamino acids, yeast ext.
  - Designed to qualitatively simulate wheat root exudates
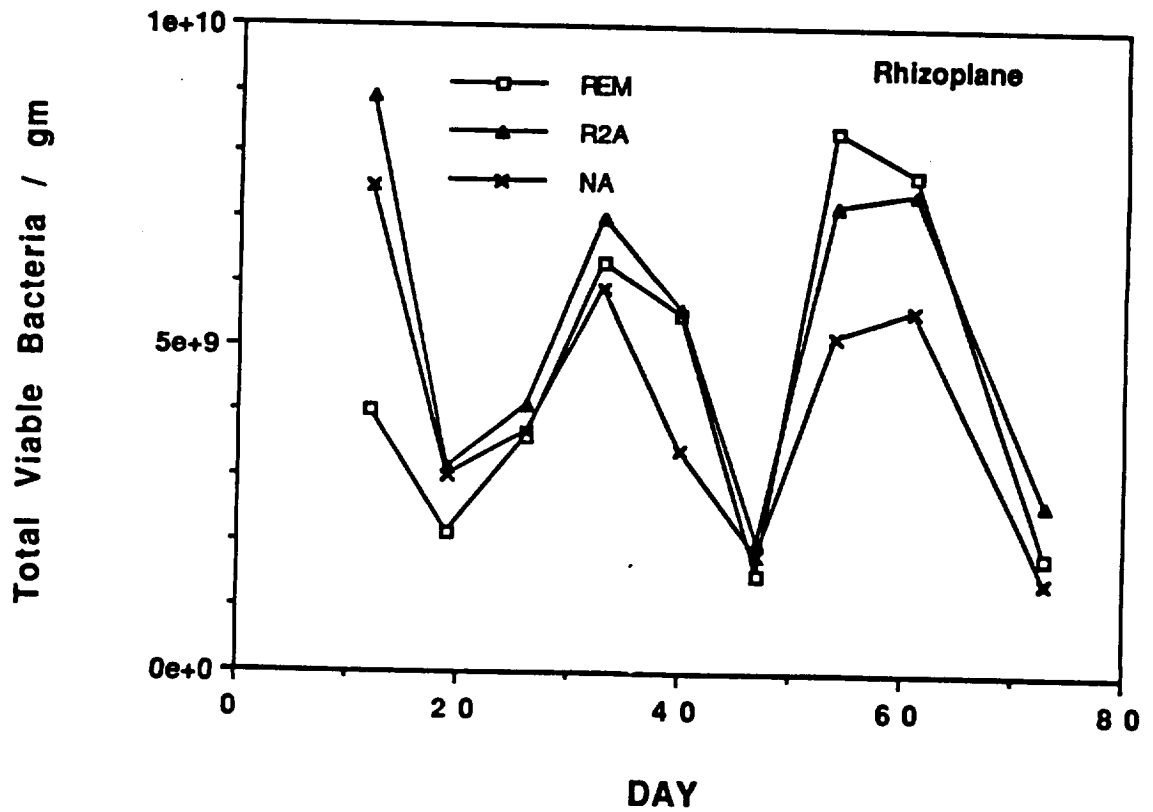
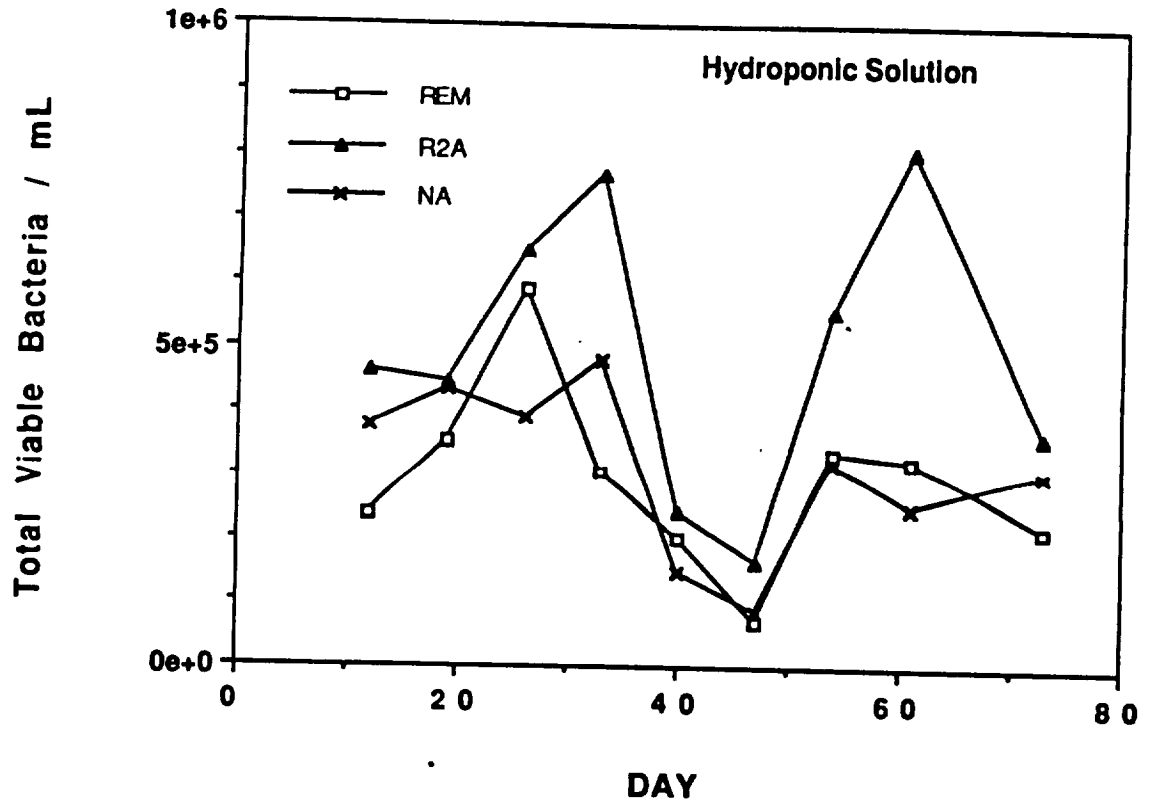15 RANDOM ISOLATES
PER COUNTABLE REM PLATE
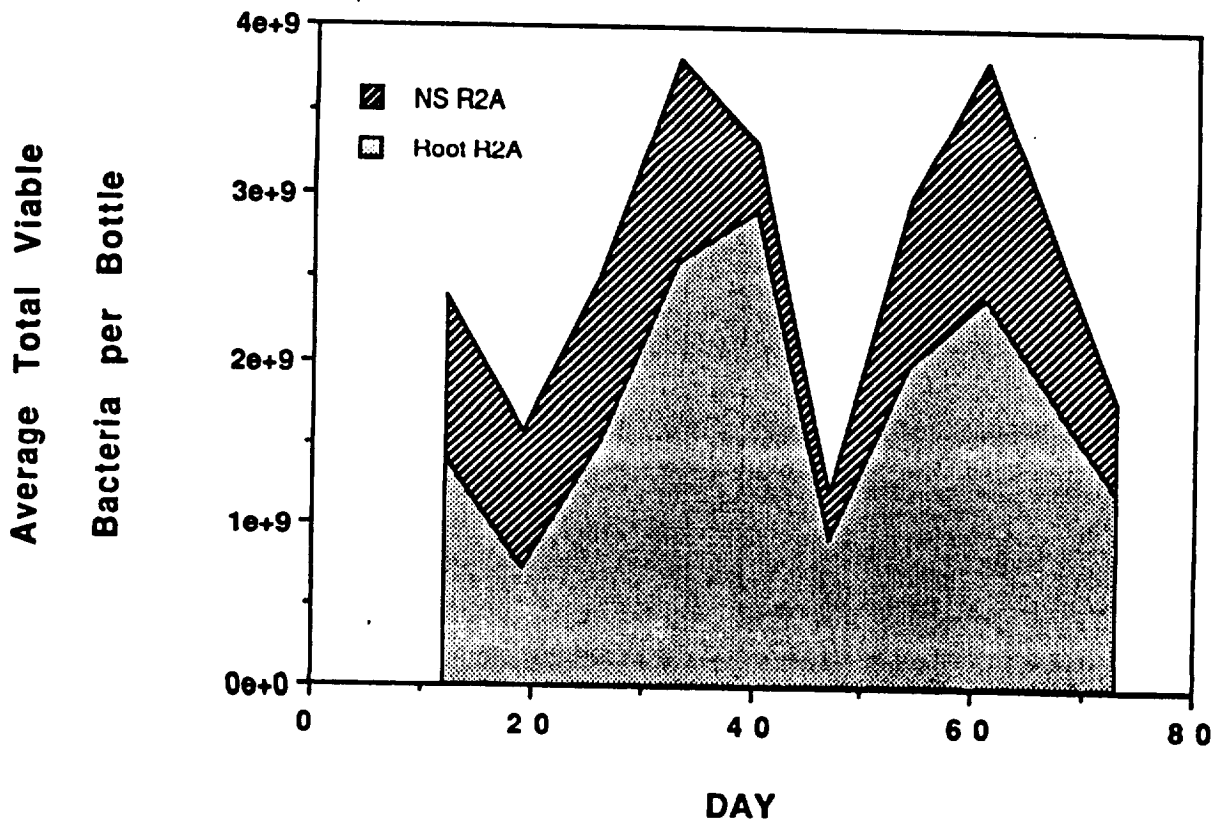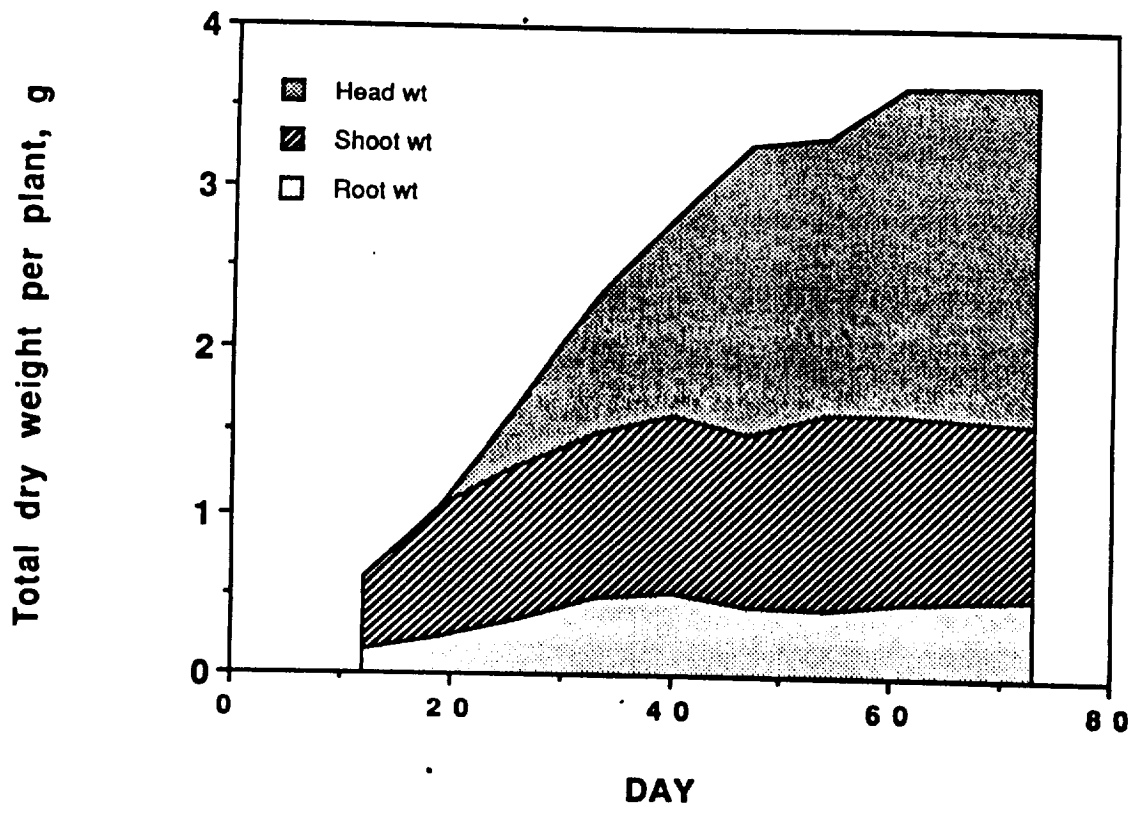
PHYSIOLOGICAL FUNCTION TESTS
- POLYMER HYDROLYSIS--
  Protein, Lipid, Plant
  polysaccharides
- GROWTH ON 2% SALT
- SOLE NITROGEN SOURCE
  - Ammonia or nitrate
- CARBON SOURCE-TTC
  REDUCTION
  - Sugars (i.e., cellobiose, arabinose, rhamnose, glucose, xylose, fructose)
  - Metabolic intermediates (i.e., acetate, succinate)

DATA ANALYSIS

- **Total Counts**
  - Two-way analysis of variance (ANOVA) with sample day and media as factors

- **Percent of isolates that were positive for physiological tests**
  - Two-way ANOVA for each test with sample day and source (suspended or rhizoplane) as factors

- **Community similarity**
  - Binary coded data for each isolate

  - Isolates from the three replicates of each day/source combination were pooled (45 total isolates)

  - Pair-wise comparisons were made between all day/source combinations

  - Clusters at the 85% level of similarity

  - Community similarity was calculated using the Sorenson index

ENUMERATION

Hydroponic Solution

Legend:
- □ REM
- ▲ R2A
- ✕ NA

Y-axis: Total Viable Bacteria / mL (0e+0 to 1e+6, marked at 5e+5)
X-axis: DAY (0 to 80)

Rhizoplane

Legend:
- □ REM
- ▲ R2A
- ✕ NA

Y-axis: Total Viable Bacteria / gm (0e+0 to 1e+10, marked at 5e+9)
X-axis: DAY (0 to 80)

Total dry weight per plant, g

Head wt
Shoot wt
Root wt

4

3

2

1

0

0   2 0   4 0   6 0   8 0

DAY

Average Total Viable Bacteria per Bottle

NS R2A
Root H2A

4e+9

3e+9

2e+9

1e+9

0e+0

0   2 0   4 0   6 0   8 0

DAY

ENUMERATION

1) Microbial densities:

- An equilibrium density was reached by the earliest sampling date:

    - Suspended: 10^5 to 10^6 CFU/mL
    - Rhizoplane: 10^9 to 10^{10} CFU/g

- These levels have been found in other crop studies with wheat, potato, and soybeans in flowing hydroponic systems, even after 200 days of continuous culture

2) Estimated bacterial load of a CELSS chamber filled with mature wheat:

BACTERIAL CONCENTRATION	X	SOLUTION VOLUME	=	TOTAL SUSPENDED BACTERIA	% OF TOTAL
(1×10^8/L)		(960 L)		(9.6×10^{10})	5

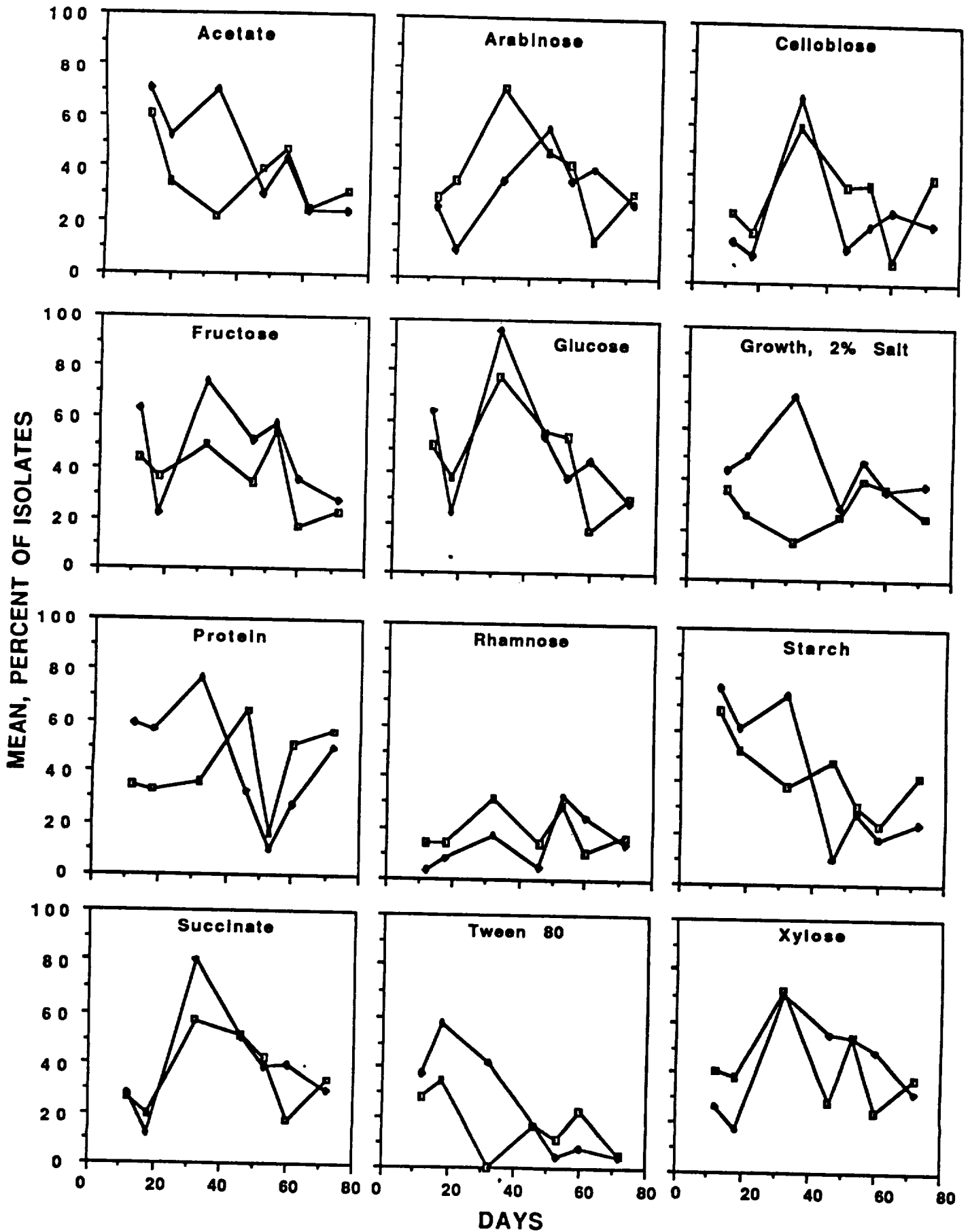| BACTERIAL CONCENTRATION	X	ROOT MASS	=	TOTAL RHIZOPLANE BACTERIA	% OF TOTAL
(1×10^9/GM)		(1920 GM)		(1.9×10^{12})	95

EFFECT OF ENUMERATION MEDIA

- Total viable counts from root samples were not significantly affected by type of enumeration media.

- Total viable counts of suspended bacteria were significantly higher (p=0.05, Duncan's) on R2A media.

  - R2A is a low-nutrient medium designed for the recovery of injured or metabolically inactive bacteria from potable water

  - Higher counts using R2A may indicate significantly higher numbers of such bacteria in the suspended population

- This result, along with the relatively low numbers of suspended bacteria, supports our working hypothesis that the nutrient solution is poor in readily metabolized organic compounds.
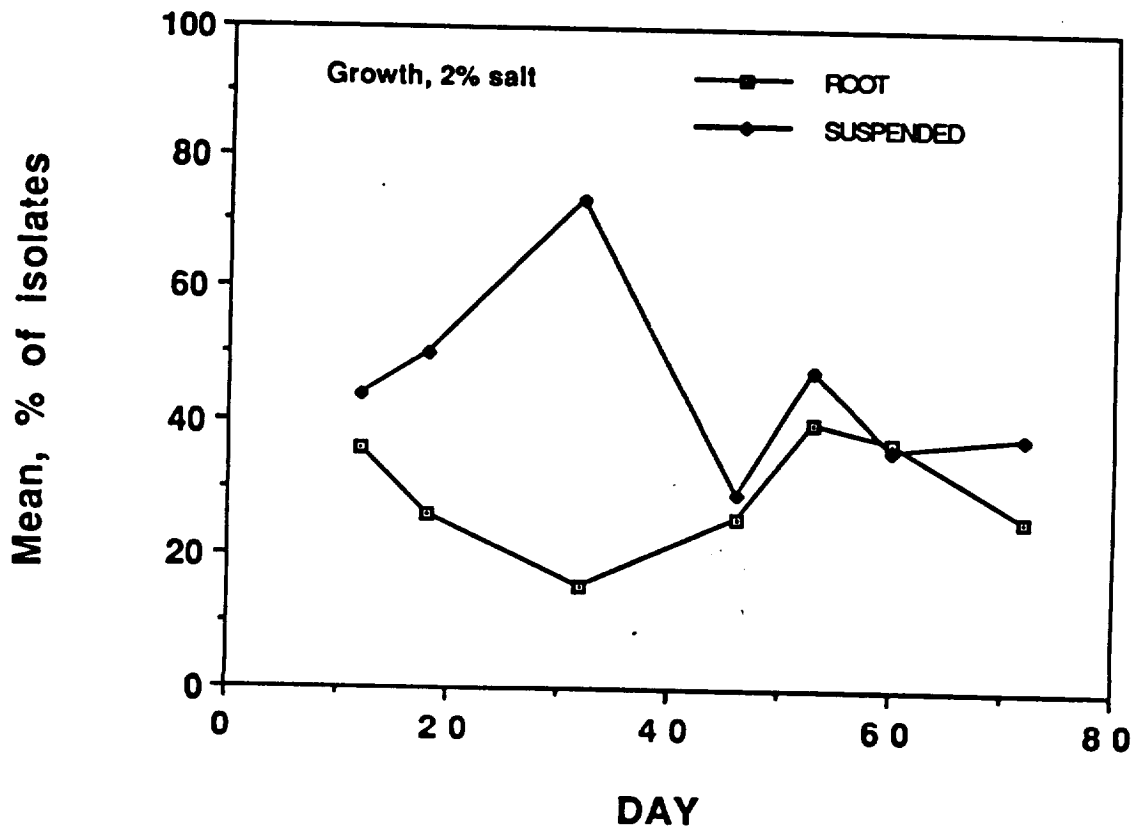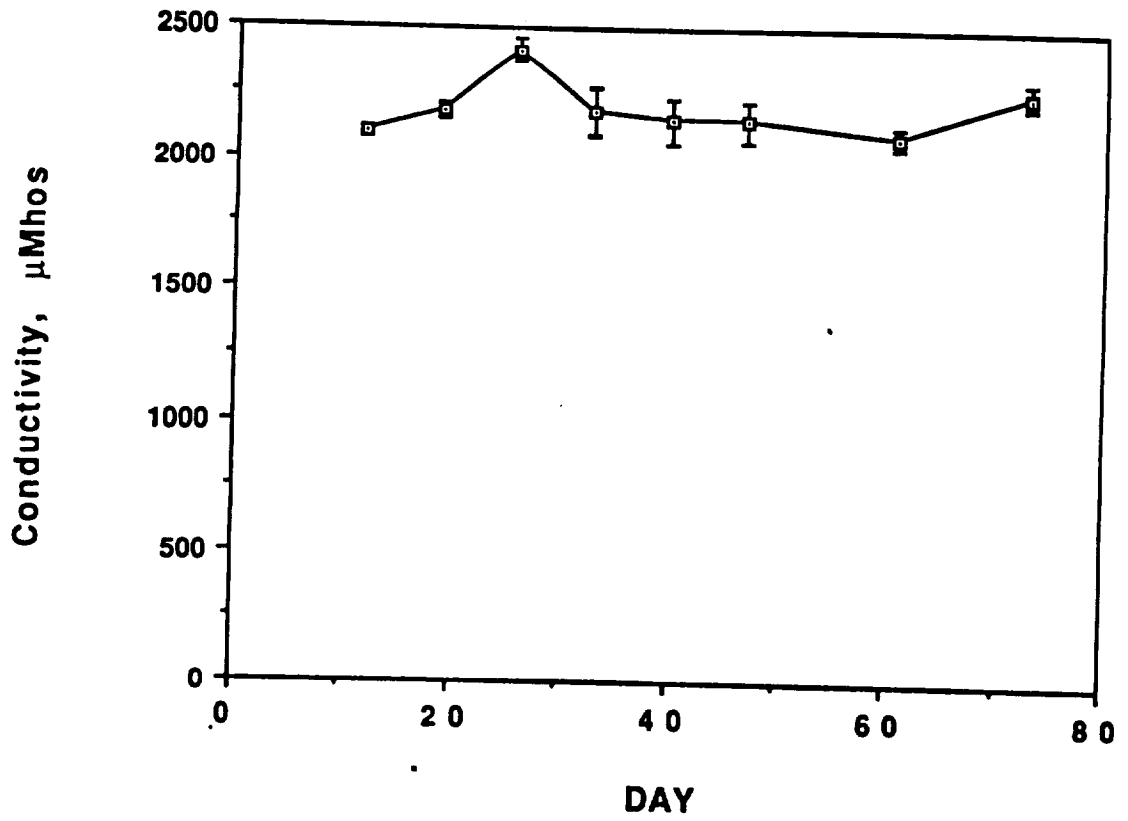
BACTERIAL FLUCTUATIONS WITH TIME

- The changes in bacterial numbers over time were significant for the rhizoplane community (p = 0.024) and close to significant for the suspended community (p = 0.064)

- Changes in the two communities seemed to follow the same trend.

    - The relationship was not significantly correlated.

    - There were a higher relative number of suspended bacteria early in the growth cycle.

- A decrease in the overall bacterial load occurred in the middle of the wheat life cycle, when the growth rate of the wheat approached zero.

    - The decrease was seen in both communities

PHYSIOLOGICAL FUNCTION TESTS

- The functional capabilities of the rhizoplane and suspended communities were similar despite changes over time.

- The percentage of isolates that tested positive for 11 of 12 tests was not statistically different between the suspended and rhizoplane communities.

- A significant source effect was present only for growth on 2% salt.

  - Largely a result of a higher percentage of salt tolerant bacteria on day 32 in the suspended community.

  - Corresponds to an increase in solution conductivity which resulted from a volume drawdown before replenishment began.

- 7 of the 12 physiological function tests were significantly different with time.

Acetate Arabinose Cellobiose
Fructose Glucose Growth, 2% Salt
Protein Rhamnose Starch
Succinate Tween 80 Xylose

MEAN, PERCENT OF ISOLATES

DAYS

SORENSON'S SIMILARITY INDEX

Day	Source	Between source within day	Between source between day	Within source between day
12	Suspended	56	30	22
	Rhizoplane		35	37
18	Suspended	46	40	30
	Rhizoplane		35	44
32	Suspended	46	20	22
	Rhizoplane		43	40
46	Suspended	56	47	37
	Rhizoplane		44	47
53	Suspended	49	33	32
	Rhizoplane		41	52
60	Suspended	56	51	46
	Rhizoplane		37	33
72	Suspended	67	41	35
	Rhizoplane		31	42

- Consistent level of similarity between the suspended and rhizoplane communities at each sampling day over the growth cycle of wheat

  - This level is greater than the similarity within or between the rhizoplane or suspended communities over time.

CONCLUSIONS

1) Rhizoplane bacterial numbers are 20 times greater than numbers of suspended bacteria in hydroponic systems.

2) A greater percentage of apparently stressed bacterial cells and a relatively low overall density in suspension indicates that the nutrient solution of hydroponic systems is not a highly favorable bacterial habitat.

3) Changes in the density and composition of the suspended bacterial community follows changes in the rhizoplane community. Changes in the rhizoplane community may be detected by monitoring the more easily sampled suspended community.

4) The composition of the suspended community is a result of inoculation from the root and differential rates of growth and death. Further study is needed to determine the relative importance of these two processes.