The author wishes to acknowledge the kindly suggestions of Mr. C. W. Brown whose sympathetic supervision has been of great assistance in this investigation.

VIABILITY OF PSEUDOMONAS RADICICOLA UNDER AEROBIC AND PARTIAL ANAEROBIC CONDITIONS.

By

F. O. Ockerblad

June, 1916.

THESIS

TABLE OF CONTENTS.

INTRODUCTION AND PURPOSE.

The work with Ps. radicicola up to the present time deals largely with the relation of the organism to its host plant; whether it is a parasite or a beneficial intruder, and with its mode of entrance into the plant. Another side of the problem which has been studied to a considerable extent of recent years is the study of the organism in the soil. The thermal death point has been determined, but it has not been shown whether the various strains have a common or a different thermal death point.

It is not the intention of the author to give an exhaustive study of Pseudomonas radicicola under various conditions but rather to present the results of his work on the viability of different strains in culture bottles under aerobic and partial anaerobic conditions, on their toleration of acids and of alkalis and on their thermal death point.

LITERATURE REVIEW.

A review of the literature upon Ps. radicicola shows that comparatively little work has been done with its viability. Harrison and Barlow(6) show that, after being grown on a medium consisting of ash-leachings, maltose, potassium phosphate and agar for a short time at 20° to 25°C. and kept at room temperature, Ps. radicicola remains alive

for nearly two years. Edwards(3) carried on a similar experiment growing the organism on ash-maltose agar in a darkened closet at laboratory temperature. He found that out of the nineteen strains used in the experiment fifteen were alive after four years. Kruijff(8) and Chester(2) state that the organism will not withstand drying on cotton. Edwards and Barlow(4) tested the resistance of the nodule-forming bacteria to desiccation on seeds -- peas, beans and red clover. It was found that the organisms died quite rapidly so that after fourteen days a small percentage were alive. They also demonstrated that the organism would not survive desiccation for more than twenty-four hours on filter paper and would succumb in less than twenty-four hours on glass. Giltner and Langworthy(5) have similar data regarding its ability to withstand desiccation on glass.

The optimum temperature for this organism has been given by several authors at different times. One of the most recent to work on this problem is Zippel(13) who states that the optimum temperature is from 18° to 20°C. He reports that the organism will not grow below 3°C. nor above 45°C. According to deRossi(11), however, it will grow slowly at 4° to 6°C., but ceases growth altogether at 0° to -1°C. and at 37° C. Beyerink(1) obtained his first negative results at 47°C., in contrast to Schloesing and Laurent(12) who found the maximum temperature to be 30°C. and to Maze'(9) who states the upper limit of growth is 35°C.

METHODS.

Media.

Ash-sugar solution is prepared by adding 10 gms. ordinary granulated sugar to 1000 c.c. ash leachings -- place 5 gms. wood ashes in a liter of tap water and filter through paper after 5 minutes. The mixture was boiled, filtered, bottled and sterilized intermittently in flowing steam.

Ash-sugar agar is prepared the same as the liquid medium except that 10 gms. agar is added and digested during the boiling.

Ashby's solution is prepared according to the following formula:

Water (distilled)	1000 c.c.
K_2HPO_4	0.2 gms.
$MgSO_4$	0.2 gms.
NaCl	0.2 gms.
$CaSO_4$	0.1 gms.
$CaCO_3$	5. gms.
Mannit	10. gms.

The mixture was boiled, filtered, tubed and sterilized intermittently in flowing steam.

Ashby's agar as prepared by adding 15 gms. agar to the solution and digesting by boiling. It was then tubed and sterilized by the Tyndall method.

Inoculation and Incubation.

The ten cultures used in this work are those employed in the distribution for legume inoculation. A suspension in physiological salt solution (about 50 c.c. of salt solution in a bulb pipette) was prepared from a two weeks old agar slant. Ash-sugar agar slants and ash-sugar solutions (about 30 c.c. in 2 oz. bottles) were inoculated with three to five drops of the suspension and incubated at room temperature (20° to 23°C.). Thirty-two bottles of each medium was inoculated with each or the ten cultures.

Other Treatment, Analysis and Determinations.

The cultures were prepared for this experiment after two weeks incubation by replacing the cotton plugs in one-half the number of both the liquid and the solid cultures with cork stoppers which had been soaked previously in a 1-1,000 solution of mercuric chloride and dried, then flamed at the moment of insertion.

Living organisms. At intervals of ten days one bottle from each set of conditions was analysed. In the case of the agar cultures the entire growth was washed off into 100 c.c. sterile physiological salt solution. With the liquid cultures one cubic centimeter was withdrawn and placed in 99 c.c. of sterile salt solution. Higher dilutions were made and plated at once in ash-sugar agar.

Total numbers. The total numbers were determined by use of the Thoma haemocytometer using the liquid cultures direct and the first suspension from the agar cultures.

Toleration of acids and alkalis. Ashby's agar was prepared and titrated with methyl red to determine the acidity. Sterile normal acetic acid or sodium hydroxide was added to the sterile agar by means of a pipette calibrated to deliver one hundred drops per cubic centimeter until the desired amount of acid or alkali was present. The medium was then melted and poured into sterile petri dishes. After it had solidified the bacteria were transplanted on the surface by making a stroke with a platinum needle. Those strains capable of making a perceptible growth are recorded positive for that reaction.

Thermal Death Point. The thermal death point of a 48 hour culture in Ashby's solution was determined by the capillary tube method. The exposure was for ten minutes at definite temperatures, then they were cooled immediately. The contents of the tubes were placed on Ashby's agar slants and recorded positive for that temperature if growth characteristic of Ps. radicicola occurred.

EXPERIMENTAL WORK.

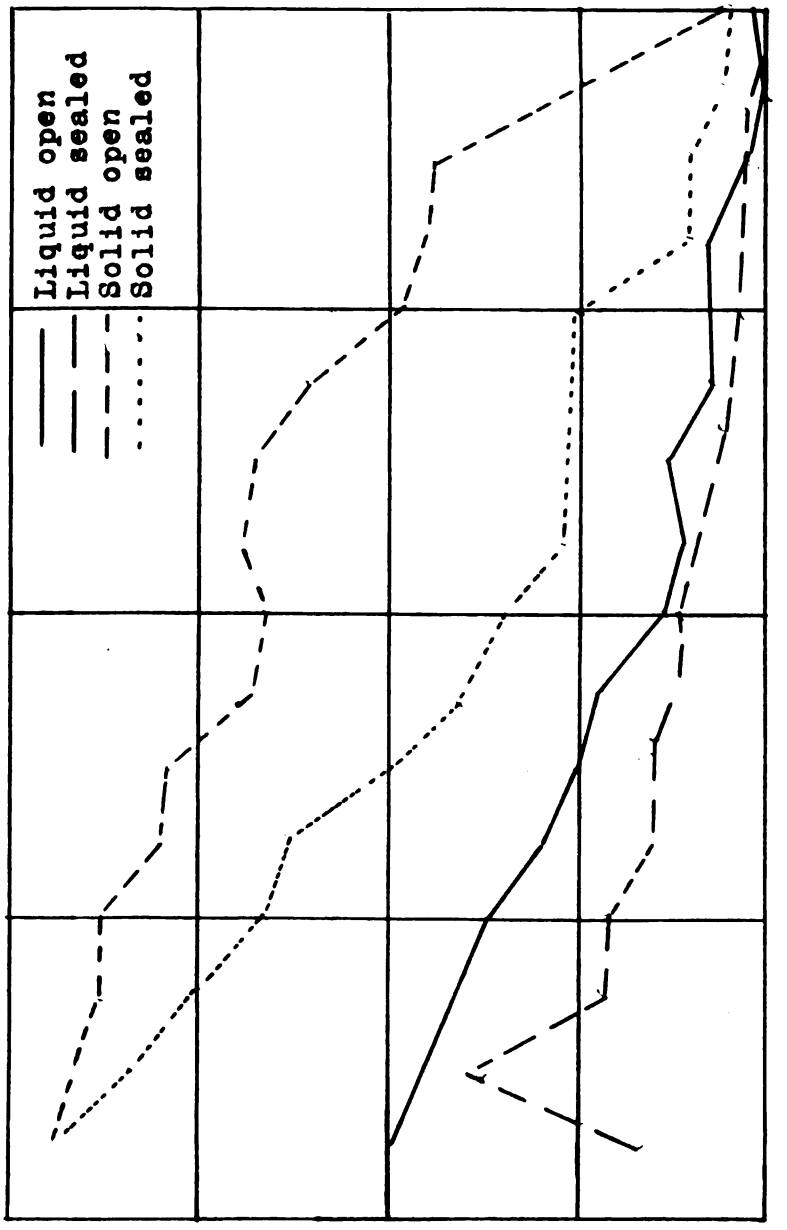
Attention is called to the plan of the investigation: The liquid and the solid media (ash-sugar solution and ash-sugar agar) inoculated with a suspension of the bacteria and incubated at 20° to 23° C. for two weeks were prepared for the experiment by replacing the cotton plugs in one-half of each of the liquid and the solid cultures with cork stoppers. The cultures remained at a temperature of 20° to 25° C. in a dark room where electric light was used occasionally until they were used for analysis. We have, therefore, for each strain of Ps. radicicola studied four sets of conditions; (1) liquid cultures under cotton plugs; (2) liquid cultures under cork stoppers; (3) solid cultures under cotton plugs; and (4) solid cultures under cork stoppers. At intervals of ten days one culture from each set of conditions was removed and analysed. Our purpose for giving two weeks incubation was to approximate commercial methods. In commercial work the cultures are grown under cotton plugs which are replaced with flamed corks just before sending out. It is desirable to know how rapidly the organisms died off in the cork stoppered bottles in order to obtain an idea as to how long the cultures may be considered good.

TABLE I

ALFALFA

Age of Culture	LIQUID						SOLID					
	OPEN			SEALED			OPEN			SEALED		
	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living
Days												
10	18,000	8,280	50	43,000	8,150	18	360,000	340,000	94	720,000	685,000	95
20	16,000	733	45	24,000	9,786	40	410,000	407,750	92	310,100	251,100	84
30	88,000	36,500	41	15,200	344	22	362,500	320,000	88	1120,000	858,800	76
40	40,000	15,000	37	26,000	5,560	21	400,000	352,000	88	700,000	465,000	66
50	52,500	18,000	30	56,000	13,000	15	456,000	368,000	80	320,000	206,000	54
60	20,000	5,066	25	29.500	411	15	120,000	84,000	79	260,000	130,000	50
70	25,000	597	23	7,600	876	12	480,000	331,000	68	500,000	200,000	40
80	25,000	4,693	14	8,000	1,090	12	400,000	266,000	66	280,000	110,000	35
90	13,200	1,550	11	28,000	2,351	9	658.250	320,000	69	480,000	142,500	27
100	12,000	1,820	13	30,000	223	7	640,000	637,250	68	500,000	121,359	26
110	8,800	673	8	404,000	26,000	6	1224,000	833,340	61	280,000	751,500	26
120	28,500	2,438	8	25,000	124	4	105,000	52,010	49	260,000	69,000	26
130	26,000	2,280	8	45,000	2,240	4	191,000	90,000	45	254,800	36,800	10
140	36,000	130	3	14,200	436	3	280,000	125,000	44	254.800	36,800	10
150	32,000	2,890	19	140,000	31	2	100,000	25,010	25	340,000	20,500	6
160	25,000	635	2	30,000	76	.2	225,000	8.500	6	360,000	19,186	5

Numbers refer to thousands



Bacteria for Alfalfa.

TABLE II

SWEET CLOVER

AGE of Culture	LIQUID						SOLID					
	OPEN			SEALED			OPEN			SEALED		
	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living
Days												
10	28,000	10,500	38	28,000	800	28	160,000	150,000	93	640,000	563,000	87.9
20	87,400	16,500	28	156.000	30,920	19	1080,000	1000,000	92	360,800	290,000	85
30	71,000	16,500	23	17,600	2,000	12	280,000	265,707	90	560,000	399,000	71
40	25,000	5,700	22	34,000	4,000	11.7	192,000	170,000	88	440,000	300,000	69
50	14,000	2,013	14.3	28,000	2,750	9	960,000	769,000	81	140,000	90,000	64
60	16,000	2,000	12.5	45,000	2.260	5	120,000	96,000	80	648,000	346,000	53
70	21,000	2,390	11	34,000	1,250	3.6	300,000	220,000	73	250,000	129,500	51
80	26,000	2,935	11	32,000	580	1	120,000	77,500	65	387,000	175,000	45
90	14,000	1,272	9	33,500	500	1	960,000	520,000	54	246,000	95,000	38
100	32,000	2,250	7	20,800	239	1.1	244,000	128,000	52	291,000	100,210	34
110	32,000	2,257	7	38,000	510	1.5	160,000	84,000	52	480,000	138,500	28
120	9,600	520	5.	429,500	124	.4	200,000	98,000	49	360,000	98,520	27
130	40,850	2,014	4.9	80,000	185	.2	431.200	190,000	44	160,000	27,500	17.1
140	20,000	375	1.8	32,000	96	.2	480,000	208,660	43	320,000	61,360	10.9
150	30,500	1,225	.4	26,000	184	.06	340,100	125,000	36	280,000	7,083	2.6

Numbers refer to thousands

Bacteria for Sweet Clover.

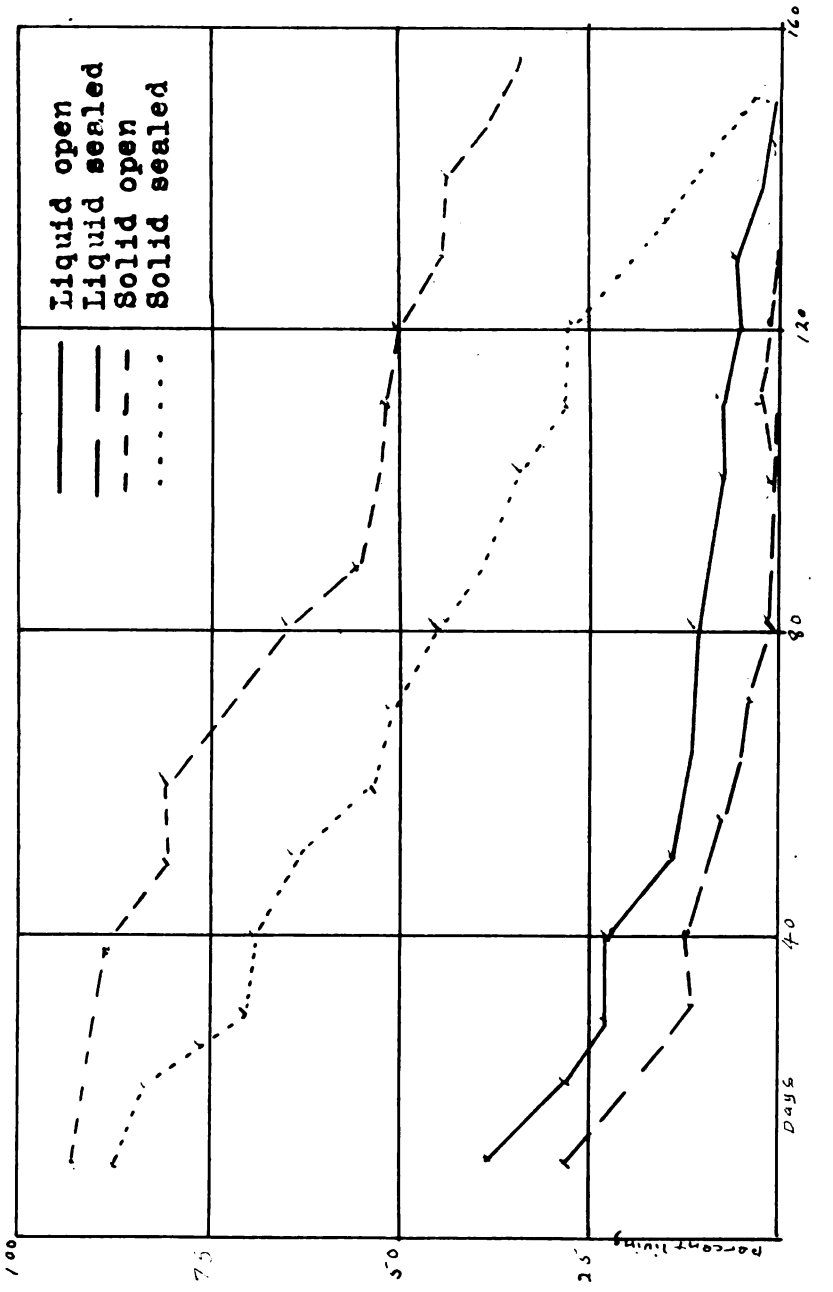
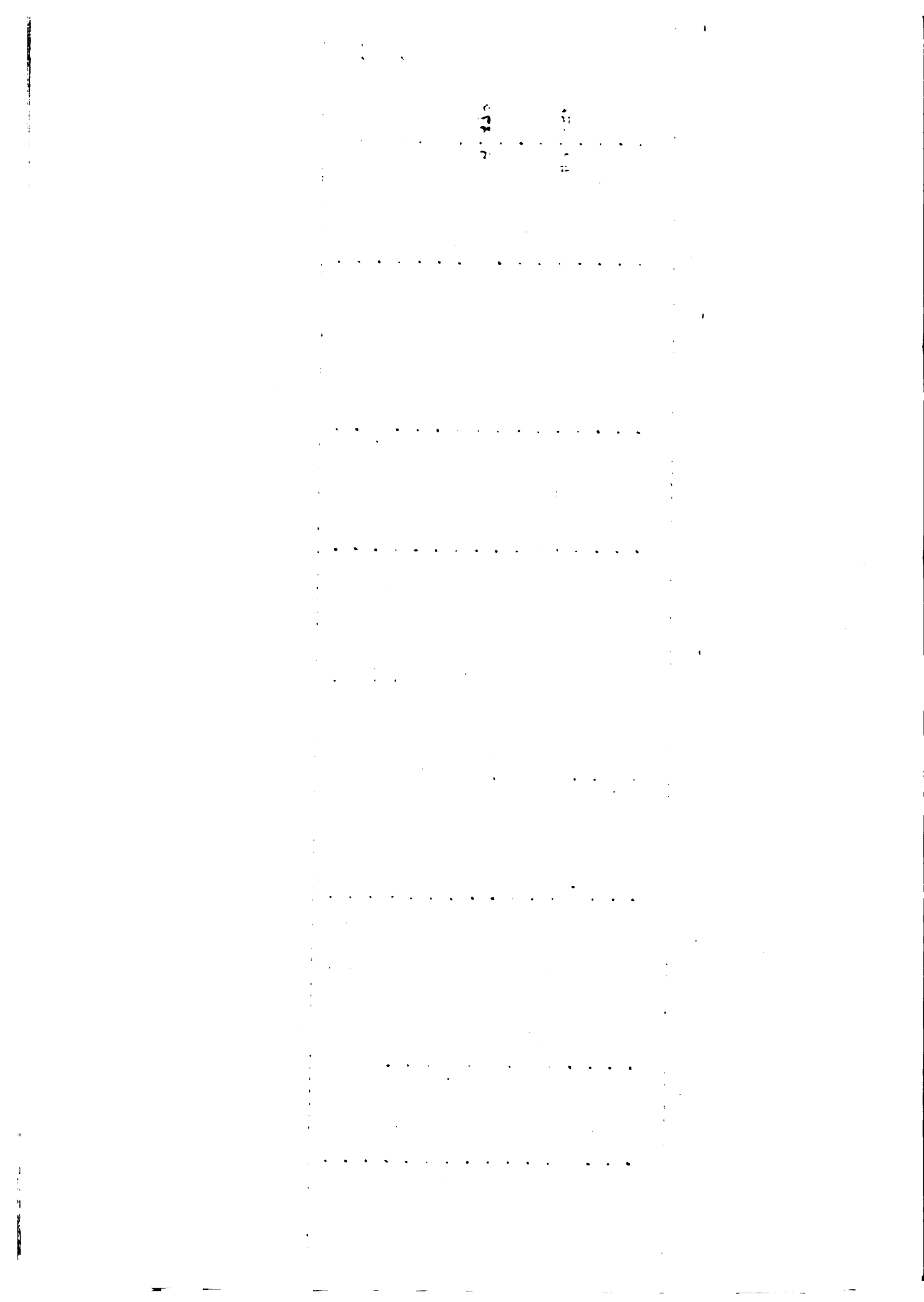


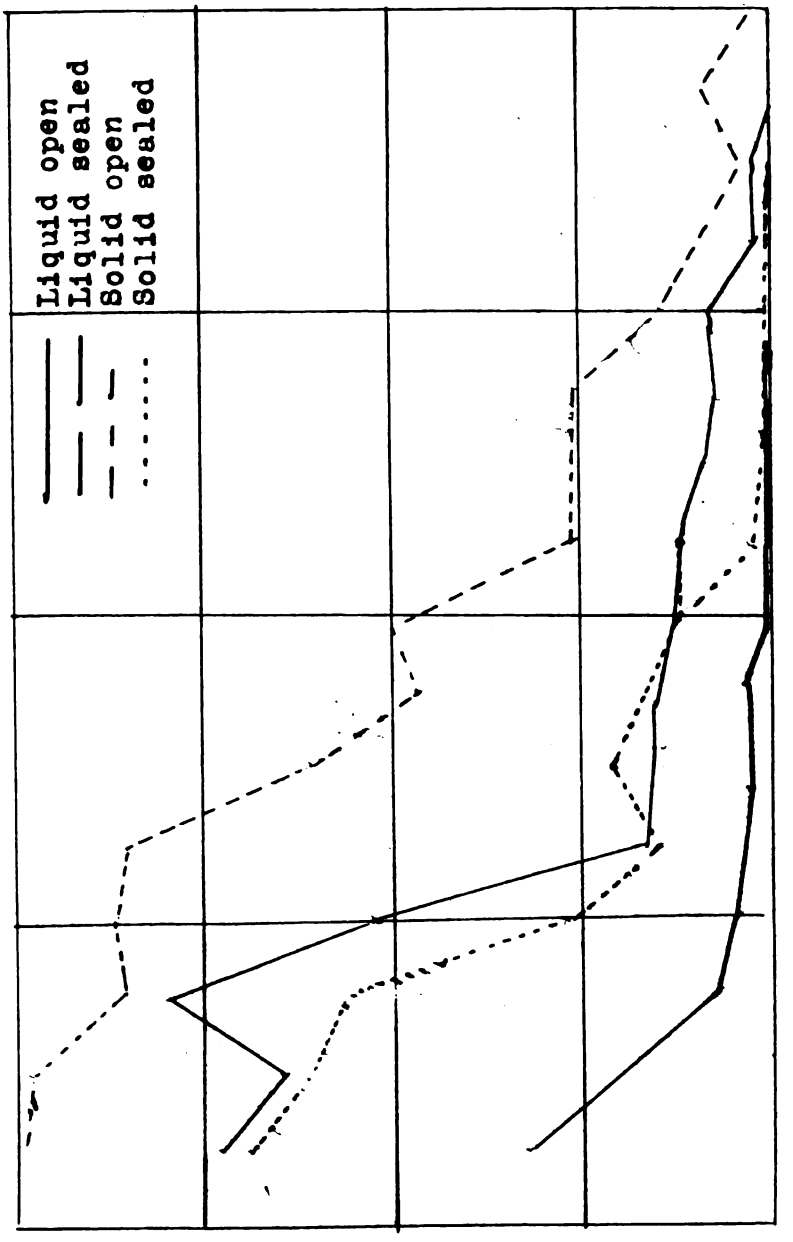
TABLE III

RED CLOVER

Age of Culture	LIQUID						SOLED					
	OPEN			SEALED			OPEN			SEALED		
	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living
Days												
10	15,000	11,637	75	8,000	2,650	33	200,000	199,000	99	360,000	251,000	69
20	22,000	14,350	65	33,600	7,172	21	40,500	40,000	98	280,000	175,000	62
30	15,000	12,000	80	24,000	2,036	8	240,000	207,250	86	280,000	160,000	57
40	20,000	10,625	53	845,000	46,000	5	115,000	100,000	87	304,000	85,000	27
50	30,000	5,386	17	81,000	4.350	5	175.000	150,000	85	280,000	42,536	15
60	36,000	5.500	15	16,000	704	4	80,000	50,000	62	150,000	32,000	21
70	260,000	40,000	15	16,000	674	4	198,000	95,5000	48	600,000	111,000	17
80	36,000	5.206	13	80,000	1,066	1	210,000	105,500	50	320,000	44,500	13
90	24,000	3,120	13	39,000	583	1	620,000	175,000	27	144,000	5,378	3
100	21,000	2,043	9	28,000	439	1	160,000	62,000	26	250,000	24,852	9
110	40,000	3,585	8	45,000	450	1	40,000	11,838	27	120,000	2,500	2
120	13,000	1,323	9	40,000	505	1	200,000	30,935	15	320,000	7,470	2
130	30,530	1,162	3	23.500	156	.6	200,000	21,000	10	288,000	1.785	.6
140	28,000	912	3	10,000	41	.1	192,200	10,000	5	400,000	2.000	.5
150	124,000	797	.6	51,000	75	.1	250,000	24,852	9	240,000	1.215	.5
160	24,000	285	.2	40,000	75	.1	250,000	10,000	4	151,000	288	.1

Numbers refer to thousands





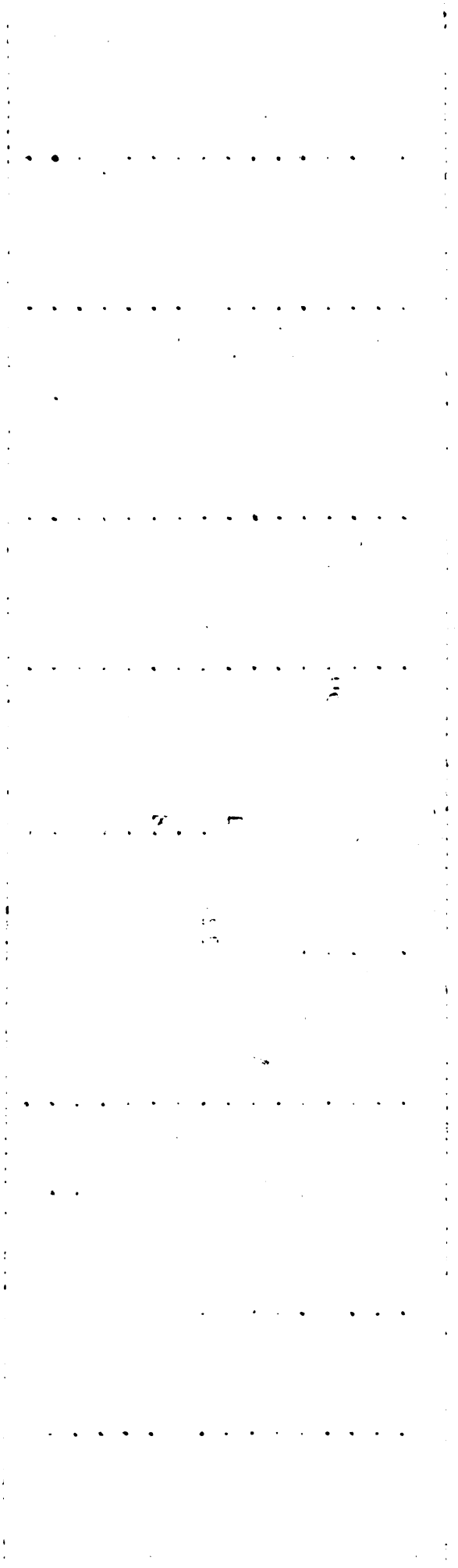
Bacteria for Red Clover.

TABLE IV

ALSIKE CLOVER

AGE of Culture Days	LIQUID OPEN Total	LIQUID OPEN Living	LIQUID OPEN % Living	LIQUID SEALED Total	LIQUID SEALED Living	LIQUID SEALED % Living	SOLID OPEN Total	SOLID OPEN Living	SOLID OPEN % Living	SOLID SEALED Total	SOLID SEALED Living	SOLID SEALED % Living
10	21,824	15,000	68	27,600	27,600	33	1100,000	1061,000	96	800,000	600,000	75
20	8,000	1,045	20	160,000	24,200	15	100,000	87 ,660	87	510,000	300.505	58
30	35,500	7,3000	20	10,240	1,043	10	320.500	280,000	87	400,000	185,735	46
40	24,000	3.512	14	30,000	2,219	7	150,000	109,000	79	480,000	124,170	25
50	10,400	1,045	10	68,000	4.346	6	260,000	200,000	76	720,000	174,375	22
60	26,000	1,832	7	81,600	909	1	410,000	300,000	73	128,000	25,887	20
70	80,000	6.250	7	56,000	861	1	150.500	105,000	69	288,000	50,567	17
80	8,000	440	5	33,000	381	1	628,000	416,000	66	600,000	77,000	12
90	92,000	1,340	4	13,600	121	.8	160,000	85,600	53	360,000	44,950	12
100	16,000	685	2	16,000	134	.8	80,000	42,000	52	28,000	3,527	12
110	20,000	497	2	23,500	200	.8	288,000	136,500	47	400,000	38,435	9
120	36,000	640	1	36,000	180	.5	256,000	115,600	45	256,000	14,647	5
130	45,000	490	1	45,000	268	.2	256,000	89,330	34	440,000	17,000	4
140	30,000	190	.9	42,000	111	.1	400,000	116,670	29	400,000	15,003	3
150	15,600	622	.3	70,000	108	.1	200,000	57,330	28.6	336,000	7,820	2
160				32,000	51	.1	275,000	77,770	28	306,000	7,880	2

Numbers refer to thousahds



Bacteria for Alsike Clover.

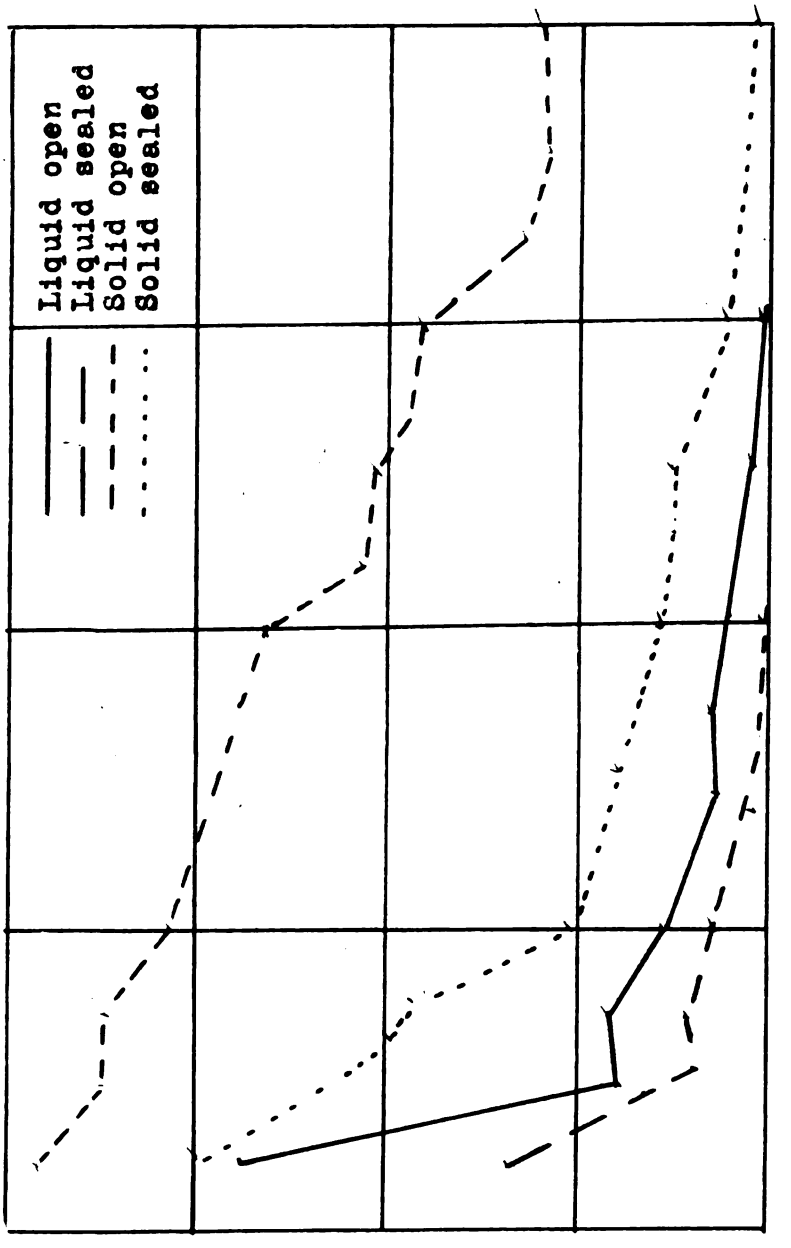
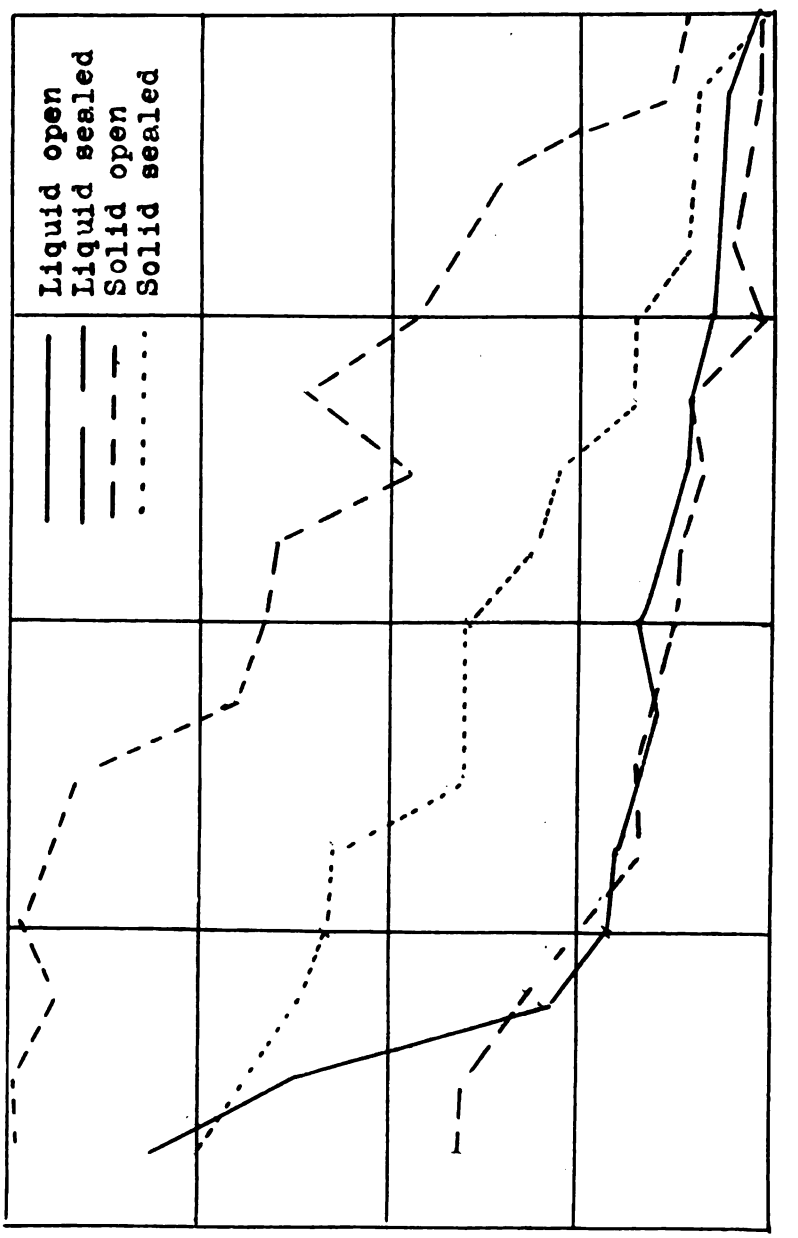


TABLE V

WHITE CLOVER

Age of Culture	LIQUID						SOLID					
	OPEN			SEALED			OPEN			SEALED		
	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living
Days												
10	50,000	41,035	82	48,000	19,996	41	507,000	503,000	99	520,000	400,000	76
20	19,140,	12,171	63	84,000	33,675	40	520,000	505,250	99	1000,000	694,000	69
30	31,000	9,633	30	30,000	10,000	33	370,000	350,500	94	280,000	175,000	62
40	31,000	6,674	21	18,000	4,337	24	675,000	662,500	98	249,200	166,500	58
50	11,200	2,258	20	2,400	400	17	160,000	132,500	89	240,000	136,931	57
60	22,000	5,430	17	39,000	673	17	231,600	200,500	86	384,000	158,000	40
70	24,000	3,820	15	30,000	6,700	15	230,000	327,500	70	192,000	77,000	40
80	14.400	2.512	17	395,000	50,000	13	215,780	144,000	66	252,000	112,500	40
90	24,000	3.360	14	60,000	5.150	12	520,000	341,000	65	550,000	172,075	31
100	32,000	380	11	6.400	620	9	516,000	250,000	48	280,000	79,975	28
110	56,000	5,600	10	34,000	3,400	10	600,000	418,750	61	280,000	51,750	18
120	26,000	2,075	8	32,000	1,015	3	525,000	250,000	47	250,000	42,660	17
130	28,000	2,125	7	32,000	1,735	5	534,250	224,000	41	410,000	55,000	11
140	45,000	3,200	7	58,500	1,988	3	615,000	210,000	34	600,000	41 ,250	10
150	36,000	2,082	6	42,000	1,200	2	452,000	62,000	13	280,000	30,000	10
160	135,000	1,600	1	36,500	366	1	448,000	48,750	10	608,000	153,300	2

Numbers refer to thousands



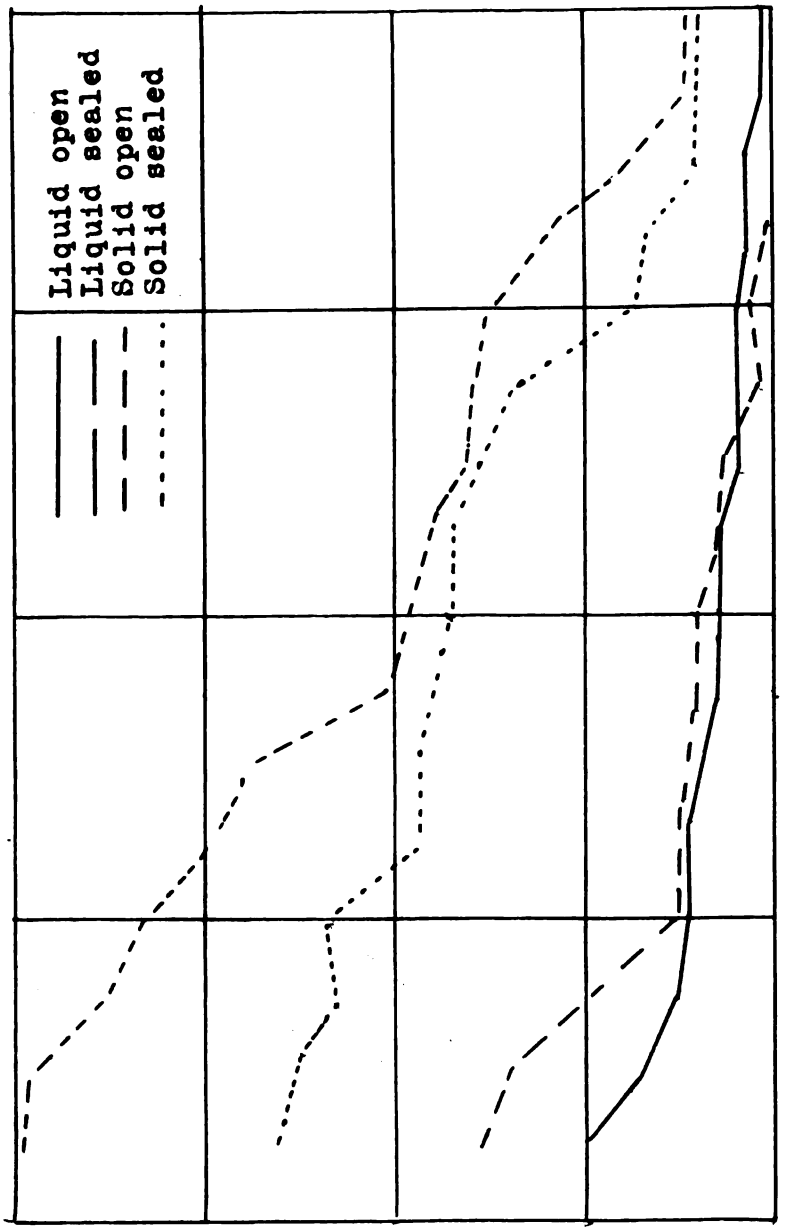
Bacteria for White Clover.

TABLE VI

FIELD BEANS

AGE of Culture	LIQUID OPEN Total	LIQUID OPEN Living	LIQUID OPEN % Living	LIQUID SEALED Total	LIQUID SEALED Living	LIQUID SEALED % Living	SOLID OPEN Total	SOLID OPEN Living	SOLID OPEN % Living	SOLID SEALED Total	SOLID SEALED Living	SOLID SEALED % Living
Days												
10	15,000	4,014	26	19,200	7,610	39	20,000	19,000	99	860,000	573,050	66
20	20,000	21,354	17	8,400	3,000	35	240,000	236,660	98	800,000	510,000	64
30	4,800	630	13	114,000	39,084	25	410,000	361,001	88	540,000	320,100	58
40	45,600	6,532	12	21,280	2,780	13	300,000	250,000	83	300,000	178,250	59
50	7,200	880	12	36,000	5,013	13	160,000	119,750	74	218,280	112,205	47
60	16,000	3,000	10	24,000	2,850	11	240,000	168,000	70	502,000	236,656	47
70	29,000	2,350	8	43,000	4,500	10	350,000	180,000	51	152,000	69,010	45
80	39,000	2,900	8	28,000	2,825	10	250,500	125,200	49	150,000	70,000	45
90	44,000	3,517	8	25,000	2,280	8	270,100	125,006	46	1200,000	519,340	43
100	22,000	1,203	5	40,000	2,500	6	255,050	105,000	41	229,640	99,250	34
110	35,000	1,928	5	27,000	643	2	200,000	90,000	40	651,000	229,000	35
120	32,000	1,714	5	58,000	1,592	3	302,000	80,000	37	209,000	40,500	19
130	25,500	1,180	4	40,000	63	1	165,000	52,000	31	288,000	45,500	17
140	50,400	2,135	4	25,600	378	1	504,000	96,000	19	680,000	80,000	11
150	30,500	680	2	10,000	206	.2	350,000	44,000	12	351,225	36,000	10
160	50,000	940	1.8	20,000	4	.02	175,000	21,000	12	160,000	25,600	10

Numbers refer to thousands



Bacteria for Field Beans.

TABLE VII

Garden Beans

Age of Culture	Liquid						Solid					
	Open			Sealed			Open			Sealed		
	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living
Days												
10	36,000	32,618	90	9,200	4,000	63	195,000	155,500	78	4,600	2,500	54
20	38,400	28,000	70	42,000	12,154	28	280,000	196,100	70	225,000	97,000	43
30	18,400	28,000	70	40,000	12,153	28	125,000	22,000	73	82,000	29,110	35
40	22,000	6,806	30	24,000	1.197	4	38,000	27,000	71	125,000	40,000	32
50	4,400	1,865	42	45,000	1,528	3	300,000	195,210	65	431,000	120,000	27
60	41,500	8,630	20	10,000	1,522	10	288,000	175,000	65	24,000	5,200	19
70	68,000	7,500	11	25,000	280	1	288,000	167,750	58	50,000	9,875	19
80	9,600	852	9	25,000	216	.8	4,600	2,500	54	261,100	52,000	19
90	44,000	3,005	6	32,000	250	.7	240,000	105,734	43	180,000	20,000	11
100	15,000	752	5	71,000	446	.6	320,000	123,206	38	40,000	2,000	5
110	26,000	1,415	5	47,000	165	.3	640,000	191,000	29	200,000	10,520	5
120	22,000	852	3	26,000	118	.4	125,000	40,000	32	250,000	8,100	3
130	30,000	1,117	3	30,000	150	.5	160,000	37,000	23	48,000	1,315	2
140	70,000	1,563	2	66,000	267	.4	95,000	20,000	21	360,000	5,317	1
150	41,510	850	2	81,000	217	.1	86,000	16,005	19	240,000	2,010	.9
160	26,010	280	1	22,000	10	.4	95,200	10,010	10	100,000	180	.1

Numbers refer to thousands

Bacteria for Garden Beans.

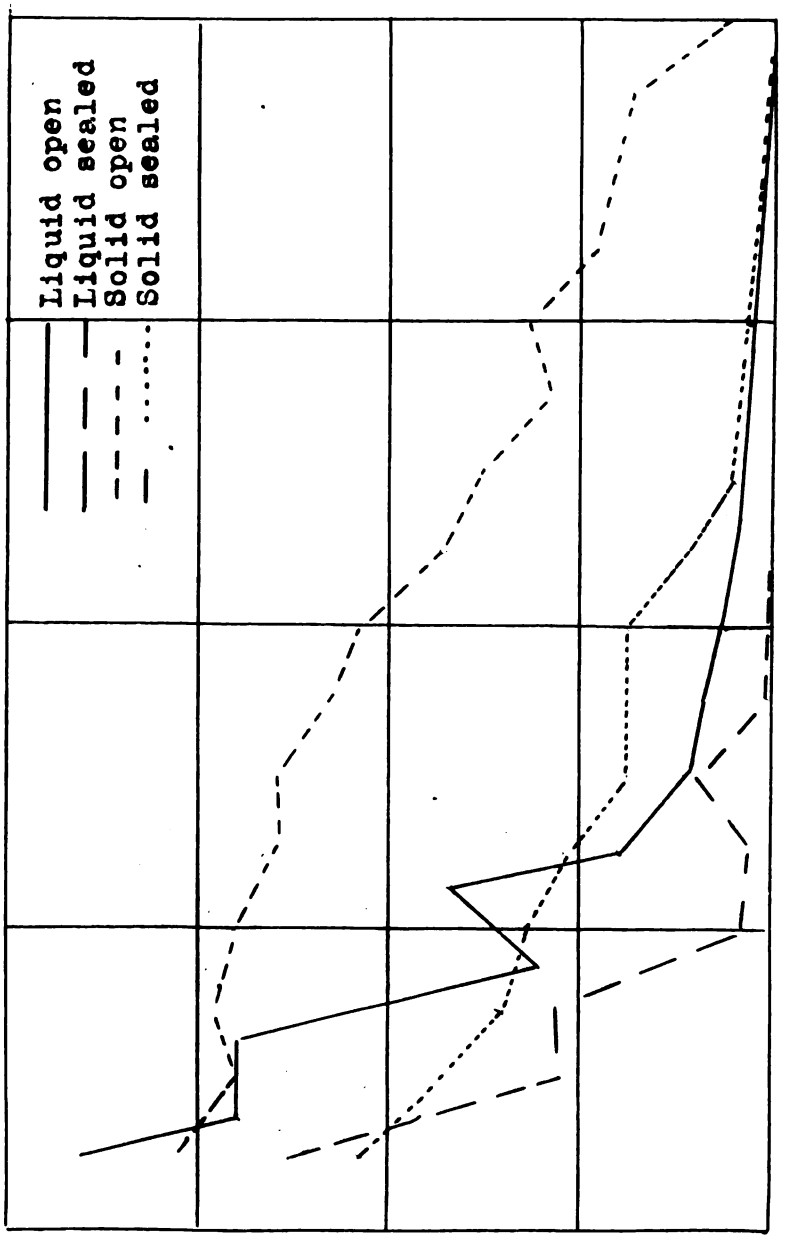
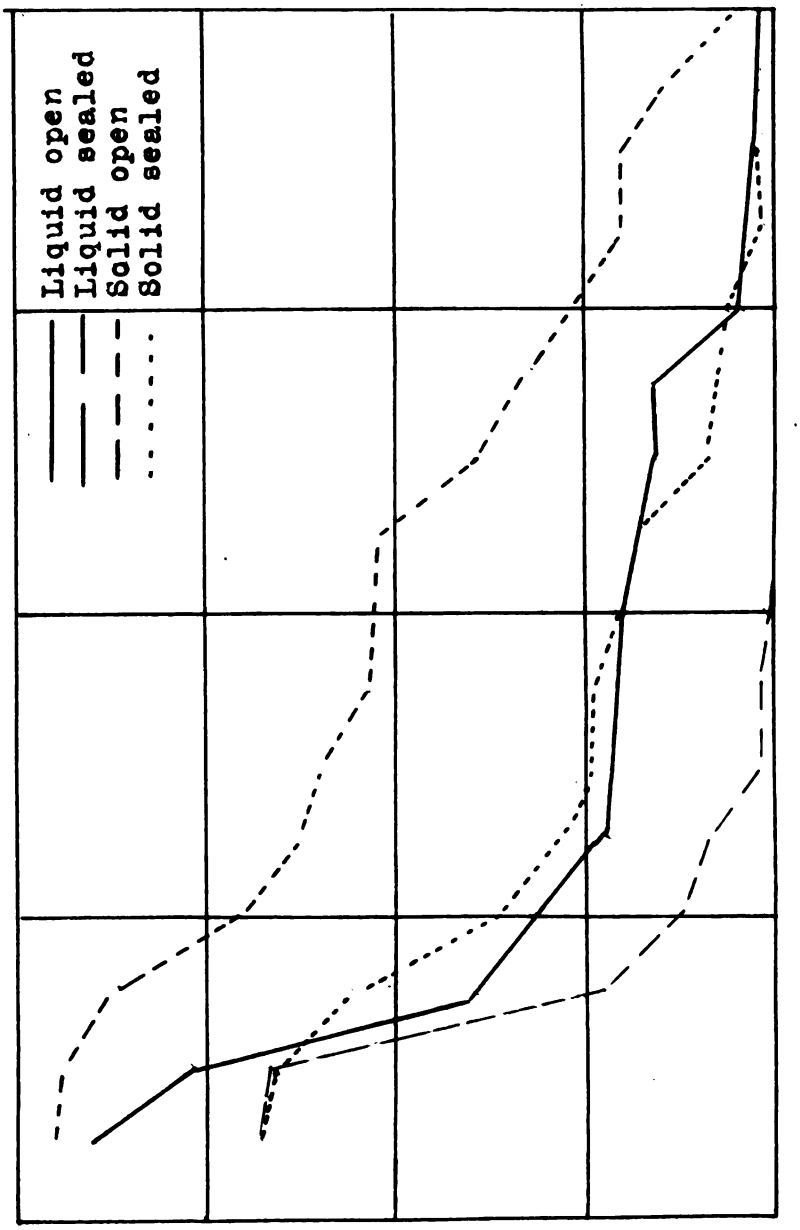


Table VIII

SOY BEANS

Age of Culture	LIQUID						SOLID					
	Open			Sealed			Open			Sealed		
	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living
Days												
10	40,000	38,000	90	8,800	6,068	68	105,000	100,000	95	560,000	38,000	68
20	12,800	10,005	77	23,000	290	67	75,000	71,000	94	160,000	100,187	66
30	7,200	2,832	39	12,000	2,782	23	136,250	120,000	88	260,200	96,005	56
40	5,200	1,705	32	16,800	2,050	12	560,000	401,250	71	300,000	110,050	36
50	19,700	4,510	23	27,600	2,582	9	164,000	104,500	63	100,000	28,000	28
60	30,000	6,870	22	6,400	133	2	412,250	250,150	60	450,000	108,500	24
70	15,000	3,247	21	49,000	123	2	260,000	141,000	54	200,000	48,843	24
80	19,000	4,152	21	47,000	810	1	160,000	85,500	53	260,000	54,458	20
90	101,000	20,00	19	20,500	104	.5	55,000	28,500	53	411,438	81,000	19
100	8,800	1,440	16	36,000	118	.3	256,000	103,900	40	200,000	18,500	9
110	18,000	3,000	16	21,000	81	.3	160,000	55,000	34	608,000	110,000	8
120	19,000	1,127	5	38,000	93	.2	200,000	53,000	26	200,000	12,200	6
130	47,500	1,960	4	61,000	86	.1	240,000	51,000	21	238,000	9,343	3
140	34,000	838	2				400,000	86,000	21	288,000	10,800	3
150	19,000	855	2	50,000	39	.07	320,000	48,000	15	240,000	6,194	2
160	50,000	1,205	2				51, 300	1,960	3	288,000	5,500	.1

Numbers refer to thousands



Bacteria for Soy Beans.

TABLE IX

GARDEN PEAS

Age of Culture	LIQUID Open Total	Open Living	Open % Living	Sealed Total	Sealed Living	Sealed % Living	SOLID Open Total	Open Living	Open % Living	Sealed Total	Sealed Living	Sealed % Living
Days												
10	16,875	10,500	62	68,500	53,200	77	245,000	240,000	98	188,000	103,670	55
20	44,000	18,000	40	26,400	11,500	43	74,000	68,666	92	150,000	67,000	44
30	45,000	16,250	36	3,000	510	17	75,000	66,000	88	560,000	165,000	29
40	22,500	6,937	30	27,200	283	10	75,000	61,454	82	360,000	99,000	28
50	17,000	5,250	30	65,500	430	6	180,000	129,000	72	240,000	56,273	23
60	17,000	4,940	29	41,600	219	5	144,000	114,250	70	520,500	103,225	19
70	17,000	5,000	29	16,000	400	3	290,000	200,000	69	102,000	61,010	19
80	48,000	10,580	22	40,600	181	4	95,000	62,005	65	160,000	26,791	14
90	40,000	8,100	22	45,000	135	3	256,000	155,500	60	256,000	32,366	12
100	49,000	7,473	11	45,000	686	1	351,000	192,000	54	310,000	32,100	10
110	17,000	2,470	14	60,000	1,001	1	120,000	58,300	50	224,000	18,220	8
120	32,000	1,524	4	46,500	41	1	200,000	52,000	26	200,000	11,000	5
130	16,000	1,000	6	20,000	261	1	200,000	55,500	22	576,000	32,000	5
140	45,000	1,827	4	26,000	228	.8	288,500	92,330	32	256,000	7,125	3
150	44,000	1,558	3	19,000	110	.5	224,000	37,000	16	280,000	4,033	1
160	61,200	1,006	1	38,000	70	.1	320,000	45,210	14	224,000	20,750	8

Numbers refer to thousands

Bacteria for Garden Pea.

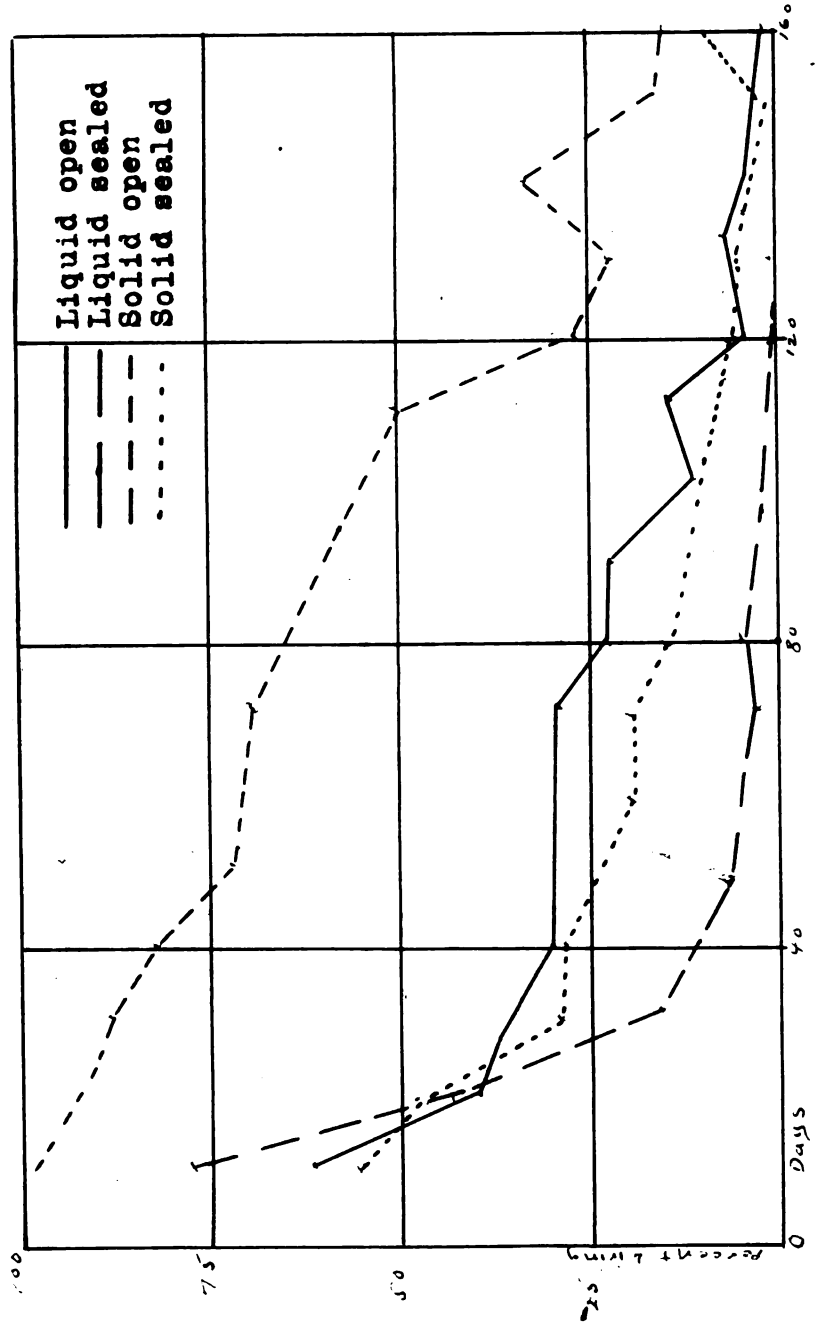


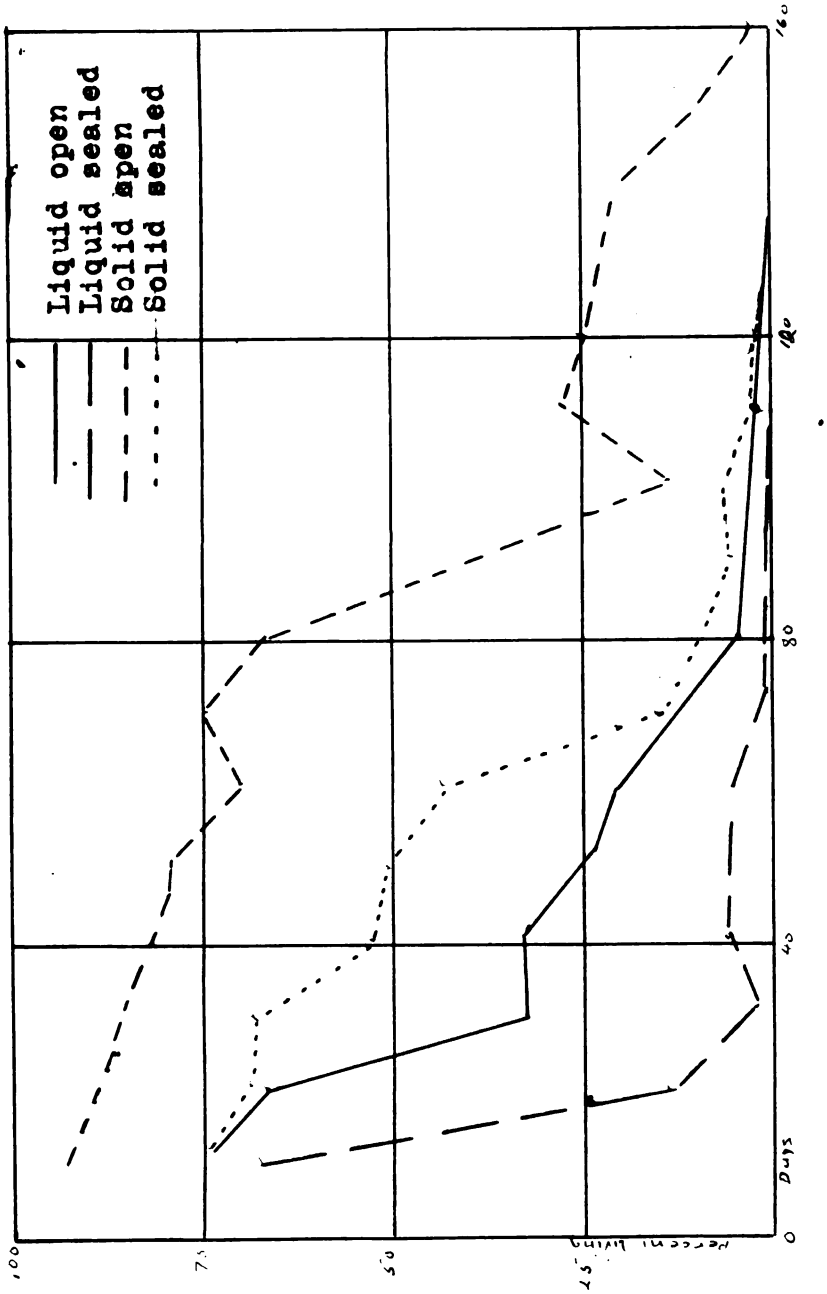
TABLE X

VETCH

Age of Culture	LIQUID						SOLID					
	OPEN			SEALED			OPEN			SEALED		
	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living	Total	Living	% Living
Days												
10	29,000	23,000	75	325,000	220,000	67	140,000	130,000	93	400,000	300,000	75
20	76,000	50,150	66	240,000	35,000	14	270,000	246,000	89	300,000	208,000	69
30	190,000	6,315	33	200,000	2,558	2	120,000	103,500	86	360,000	245,000	68
40	6,000	2,000	33	53,208	3,516	66	80,000	64,800	81	400,000	212,000	53
50	8,000	2,010	25	17,000	1,033	6	250,500	200,000	79	100,000	50,000	50
60	20,000	4,200	21	20,500	1,029	5	75,000	53, 000	70	410,000	180,000	43
70	18,400	2,087	11	41,000	1,140	2	320,000	240,000	75	200,000	29,020	14
80	40,000	1,700	4	70,000	750	1	75,000	50,333	67	304,000	27,660	9
90	33,000	1,000	3	40,000	405	1	200,000	26,262	13	356,400	24,083	6
100	16,000	570	3	11,200	95	.9	4,820	2,100	43	288,000	19,270	6
110	31,500	1,000	3	5.500	145	.4	190,000	53,500	28	206,000	5,195	2
120	37,500	1,000	2	45,000	104	.3	200,000	51,062	25	192,000	5,000	2
130	26,000	535	2	32,000	68	.2	240.000	51,500	23	360,000	2,916	1
140	45,000	527	1	31,000	43	.1	224,000	46,000	20	160,000	2,098	1
150	60,000	357	.5	50,000	67	.1	260,000	25,327	9	120,000	1,625	1
160	29,000	630	.5	70,000	68	.09	250,000	6,800	2	280,000	1,798	.6

Numbers refer to thousands

Bacteria for Vetch.



Viability in Culture Bottles.

The growth in the liquid medium was turbid throughout at first and later settled to the bottom. When this stage was reached it was difficult to distribute the organisms evenly throughout the medium as they soon settle upon standing. The growth on agar is thick, hyaline to pearly and rather well distributed. There is a much smaller number of organisms in the liquid than in the solid cultures. This was noticeable throughout the experiment. When we compare the viability of the strain for alfalfa with that for sweet clover we find that at twenty days there is 84 percent living (Table I) and 85 percent living (Table II) on ash-sugar agar under the cork stoppers. After 100 days the strain for alfalfa has 26 percent living while that for sweet clover has 34 percent. And then taking the data at 150 days we find that while alfalfa has 6 percent living, sweet clover has 11 percent In the same manner let us compare the strains for red clover and for alsike clover. We find that the sealed agar culture for red clover has 62 percent living and that for alsike clover has 58 percent after 20 days. Going on to 100 days we find that the red clover strain has 9 percent living and that the alsike clover strain has 12 percent. After 150 days we find that the red clover strain has decreased to 0.5 percent and alsike clover strain to 2 percent. Comparing the strain for white clover with that for field bean we find that 69 percent and 64 percent

respectively are living after 20 days. After 100 days we find 28 percent and 39 percent living, and at the end of 150 days both the cultures have 10 percent living. The total number in a culture of any strain of Ps. radicicola on the agar under the cotton plugs is no greater than under the cork stopper. A point of note is that the cotton plugged cultures did not die off as rapidly as the sealed cultures. The liquid cultures at all times had a smaller total number of organisms, that is, Ps. radicicola is unable to increase in 30 c.c. ash-sugar solution (about 10 square centimeters surface exposed to air) to as great a number as on an ash-sugar agar slant (about 18 square centimeters surface). The death rate -- 10 to 75 percent within 10 days -- is high in the liquid cultures.

Some of the factors which caused a decrease in the number of living organisms are, (1) partial anaerobic conditions, (2) accumulation of metabolic and toxic products, etc., (3) plasmolysis caused by the concentration of the ash-sugar solution through evaporation. In the case of the unsealed liquid cultures which constantly show a much smaller number of organisms it is probable that the partial anaerobic conditions in the medium was a factor in the decreased numbers of living bacteria. The accumulation of metabolic products, toxic products, etc., are another possible factor in reducing the living organisms although no bacteroidal forms, which are supposed to be the result of unfavorable conditions, were observed at any time. It

is of note that while Edwards(3) obtained sub-cultures after four year he did no quantitative analysis and it is possible that toxins killed four of his cultures. Plasmolysis could not be a factor in the dying off of the cultures as the rate of evaporation was quite slow (about one cubic centimeter in ten days). In the unsealed agar cultures desiccation was probably a factor in reducing the living bacteria. But with the sealed cultures of both media the partial anaerobic conditions, which tended to become wholly anaerobic, were the principal cause of the dying off of the bacteria.

Upon comparing the graphs of the different strains it will be seen that in several instances there is a sharp rise in the percent of living bacteria. This may be accounted for by the fact that a different bottle, which may have had different but unknown conditions entering, was used for each analysis. It will be observed that the total numbers are fairly constant from the beginning to the end of the experiment.

Tolerance of Acids and Alkalis.

The ashby's agar used in this work was titrated while hot with several indicators and the results recorded as follows:

Methyl red	+1.7°
Methyl orange	+2.5°
Cochineal	+1.0°
Litmus	+1.8°
Phenolphthalein	-0.5°

Table XI.

Growth on Acid and Alkaline Media.

Bacteria for	Acetic acid			Sodium hydroxide		
	20°*	10°	5°	-5°	-10°	-20°
Alfalfa	-	-	-	+	+	+
Sweet clover	-	-	-	+	+	+
Red clover	-	-	-	+	+	+
Alsike clover	-	-	-	+	+	+
White clover	-	-	-	+	+	+
Field bean	-	-	-	+	+	+
Garden bean	-	-	-	+	+	+
Soy bean	-	-	-	+	+	+
Garden Pea	-	-	-	+	+	+
Field pea	-	-	-	+	+	+
Cow pea	-	-	-	+	+	+
Vetch	-	-	-	+	+	+

*A degree is equivalent to a percent N/10.

The reaction of the tubes of medium used for the experiment was adjusted to the methyl red. It will be seen by referring to Table XI that none of the strains were able to withstand an acidity of +5° Fuller's scale, whereas all the strains were able to make a good growth in the medium with a reaction of -20°. This fact is significant in the culture of legumes since both the plants and the bacteria

Table XII.

THERMAL DEATH POINT DETERMINATIONS.

Bacteria for	Temperature of Exposure						
	58°C.	59°C.	60°C.	61°C.	62°C.	63°C.	64°C.
Alfalfa	+	+	-	-	-	-	-
Sweet clover	+	+	+	-	-	-	-
Red clover	-	-	-	-	-	-	-
Alsike clover	+	+	+	-	-	-	-
White clover	+	+	+	-	-	-	-
Field bean	+	+	-	-	-	-	-
Garden bean	+	-	-	-	-	-	-
Soy bean	+	+	-	-	-	-	-
Garden pea	+	+	+	-	-	-	-
Field pea	+	-	-	-	-	-	-
Cow pea	+	+	-	-	-	-	-
Vetch	+	+	-	-	-	-	-

require an alkaline condition for their best growth. It is probable, however, that Ps. radicicola can withstand a greater acidity in the soil than in an artificial medium since the soil particles will adsorb and the organic matter in the soil will absorb a large part of the acid. These phenomena are not pronounced in the medium used.

Thermal Death Point.

By referring to Table XII it will be noted that nine of the strains will reproduce after an exposure of ten minutes to a temperature of 59°C. While four of the twelve will stand a temperature of 60°C., none of them are able to grow after being exposed for ten minutes to a temperature of 61° C. This shows that the thermal death points of these strains of Ps. radicicola fall within narrow limits.

GENERAL DISCUSSIONS.

The mass of growth on the surface of the agar cultures is not composed entirely of bacterial cells. It is estimated that from 50 to several hundred percent of this mass is slime. In each culture there is present a number of bacteria sufficient to allow, when 60 pounds of seed is treated, several thousand bacteria per seed.

A number of experiment stations and commercial firms distribute cultures of nodule-forming bacteria in liquid media. The results obtained with ash-sugar solution are not favorable for liquid media. Even solid media, ash-sugar agar, does not keep a large number of bacteria alive for prolonged periods of time. By observing the curves in this work it is seen that the bacteria die off rapidly: At the end of thirty days the unsealed liquid cultures have an average of 38.5 percent living and the sealed liquid cultures have only 18.0 percent, while the

unsealed agar cultures have an average of 87.0 percent living and the sealed agar cultures have 55.8 percent. It, therefore, is imparative that a time limit for the use of the cultures be observed.

SUMMARY.

1. Ash-sugar agar supports a better growth of Ps. radicicola than ash-sugar solution.

2. Cultures of Ps. radicicola in cork stoppered bottles die quite rapidly: the organisms living after 160 days average 0.21 and 3 percent in the liquid and solid cultures respectively.

3. The viability in ash-sugar solution is much shorter than on ash-sugar agar.

4. On ash-sugar agar under cotton plugs the different strains of Ps. radicicola die off gradually: An average of 91.1 percent living after 20 days, of 46.2 percent after 100 days and of 12.5 percent after 160 days.

5. The strains of Ps. radicicola are sensitive to acids, that is, they fail to grow on Ashby's agar to which 0.5 percent N/1 acetic acid was added. The tolerance of alkalis is greater, that is, they make a good growth on Ashby's agar to which 2.0 percent N/1 sodium hydroxid was added.

6. The thermal death point of twelve strains of Ps. radicicola determined in Ashby's solution fall between 59° and 61° C.

Literature Cited.

(1) Beyerinck, M. W.
1890. Künstliche Infection von Vicia Faba mit Bacillus radicicola.
In Bot. Zeitg. Bd. 48, pp. 837-843.

(2) Chester, Frederick D.
1907. The effect of desiccation on root tubercle bacteria.
Del. Expt. Sta., Bul. 78, p. 15.

(3) Edwards, S. F.
1911. Viability of Ps. radicicola on ash-maltose agar.
In Science n. ser. Vol. 33, pp. 543-544.

(4) Edwards, S. F. and Barlow, B.
1909. Legume bacteria.
Ontario Dept. Ag., Bul. 169, p. 32.

(5) Giltner, W. and Langworthy, H. Virginia.
1916. Some factors influencing the longevity of soil microorganisms subjected to desiccation, with special reference to soil solution.
In Jour. Agr. Research, Vol. V, pp. 927-943.

(6) Harrison, F. C. and Barlow, B.
1907. The nodule organism of the Legumnosae,- its isolation, vultivation, identification and commercial application.
In Centrbl. f. Bakt. Abt. II, Bd. 19, pp.426-441.

(7) Hiltner, L.
1900. Ueber die Bakteroiden der Leguminosenknöllchen und ihre willkürliche Erzeugung auserhalb der Wirtspflanzen.
In Centrbl. f. Bakt., Abt.II, Bd.6, pp. 273-281.

(8) Kruijff, E. de.
1907. Onderzoekingen over - en entproeven met de z.g. Knolletsbacteriën.
In Jaarboek van net Department van Landbourv in Nederlandsh Indië; Batavia, G. Kolff and Co., pp. 112-114.

(9) Mazé, M.
1897. Fixation de l'azote libre par les bacille des nodosités des Legumineuses.
In Annales de l'Institut de Pasteur, Tm. XI, pp. 44-54.

(10) Prazmowski, A.
1889. O istocie i znaczenuin biologicznem brodanek korzeniowych grochn.
Bot. Centrbl. Bd. 39, pp. 356-362.

(11) Rossi, Gino de.
1907. Ueber die Mikroorganismen, welche die Wurzelknöllchen der Leguminosen erzugen.
Centrbl. f. Bakt. Abt. II, Bd. 18, pp.481-483.

(12) Schloesing, Th. and Laurent, Em.
1892. Recherches sur la Fixation de l'azote libre par les plantes.
In Annales de l'Institut Pasteur, Tm. VI, pp. 65-115.

(13) Zipfel, Hugo.
1912. Beiträge zur Morphologie und Biologie der Knöllchenbakterien der Leguminosen.
Centrbl. f. Bakt. Abt. II, Bd. 32, pp. 97-137.