QUARTERLY REPORT TWO

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UNPUBLISHED PRELIMINARY DATA

THE GENERAL AND COMPARATIVE BIOLOGY OF TERRESTRIAL ORGANISMS UNDER EXPERIMENTAL STRESS CONDITIONS

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I. INTRODUCTION

Quarterly Report Two continues the analysis of stress factors in seed germination and seedling growth. The results consist, in large measure, of extensions of the earlier lines of investigation.

Earlier factorial studies on anaerobic germination have been enlarged to 241 species and the entire set of results has been examined phylogenetically. Previous studies with oxidants and with simulated environments are related to the current consideration of the effects of nitrogen-oxides. The "screening" of varieties by the use of various atmospheres as selection tools continues earlier cytological and genetic observations for the purpose of selecting promising, stress-resistant breeding stocks.

Some of the studies are pointed both toward exobiological problems, and at the same time, toward potential hazards of a closed, artificial system. The nitrogen-oxides studies qualify, in this category, as do the experiments with CO_2 and volatile organic compounds.

Several other areas are under study using seeds. These are described briefly in Section VIII.

A major portion of our exploratory experimentation with seeds has been completed. More work is planned with factors such as light, nutrients, and hormones, but the principal efforts will involve (a) seedling selection and breeding for stress resistance and (b) biochemical analyses of successful and unsuccessful types, especially examination of ribonucleic acid and ribonucleoprotein fractions.

Otherwise, relatively greater attention in the immediate future will be given to the performance of more mature plants and plant organs.

II. ANAEROBIOSIS IN SEED GERMINATION: SYSTEMATIC AND PHYLOGENETIC ASPECTS

Anaerobic seed germination in 241 species has been examined. Most of these were reported in Quarterly Report One. Even though this survey, employing over 70,000 seeds, seems sizeable, it involves only about one-fifth of the known families and only 0.1% of all known species. Nevertheless, some effort has been made to determine the relationship between the distribution of the anaerobic capability and generally accepted phylogenetic organization of modern flowering plants.

For this purpose, the generally accepted ordinal system of Charles Bessey (The phylogenetic taxonomy of flowering plants. Annals. Missouri Botan. Gard., 2, 109 (1915)) has been employed. In his schema, the most primitive order, the Ranales, is a complex of plants whose floral structures have the most in common with the strobilus or cone of non-flowering seed plants. The magnolia is a prime example of the primitive condition. Several "upward" evolutionary trends are traced thence — first, fusion of many unit ovaries into a compound multi-chambered structure (polycarpy ⟶ syncarpy); second, fusion of floral parts with reduction in number and introduction of bilateral instead of radial symmetry (polypetaly ⟶ sympetaly); and third, transverse fusion of parts to form a structure within which the ovary is embedded or by which it is surrounded (hypogyny ⟶ epigyny).

From the ancestral ranalian complex, both the monocotyledonous and dicotyledonous lines were derived.

A simplified Besseyan schema is given in Figure 1. It includes all common orders, those sampled being underlined. The fractional number by each underlined order indicates its "score" for anaerobiosis. The denominators

gives the total species tested within the order irrespective of family or genus. The numerator indicates species in which some anaerobic ability exists. The major regions of the schema are indicated by letters A, B, etc.

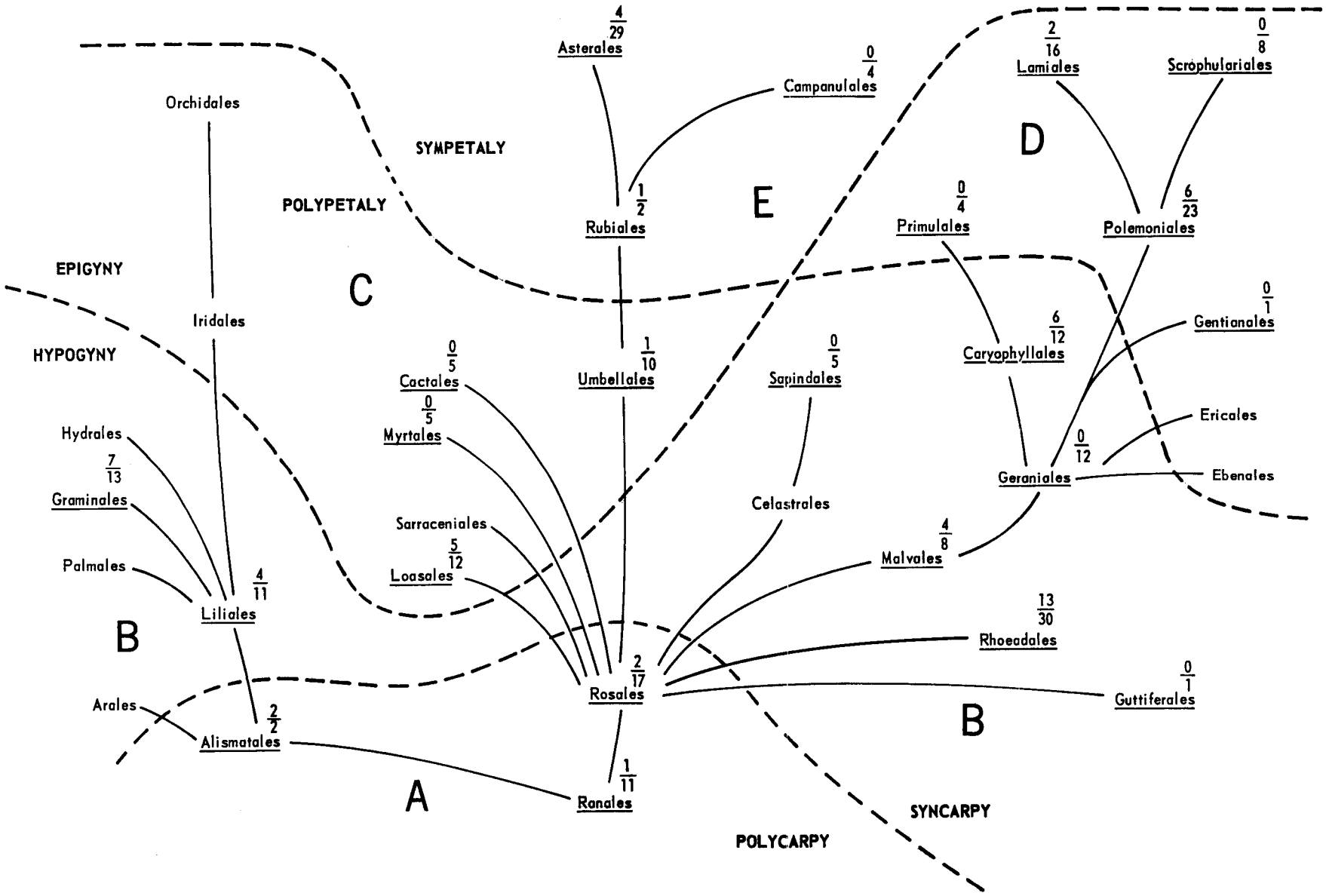

Figure 1. The Besseyan scheme of classification for flowering plants, showing the orders of the plants tested and their anaerobic performance. Orders sampled were underlined. Anaerobic species and species tested are shown respectively on the numerator and demoninator of a fraction.

In 23 orders tested, 14 contained anaerobic members. Among the 241 species, 58, or 24%, were found to be anaerobes. Among the four highest orders, 57 species yielded only 6 anaerobes, or 11%. Even the 7 highest orders, with 86 species tested, yielded only 13 anaerobes, or 15%. The three lowest orders, representing tests with 30 species yielded 5, or 16% anaerobes. Thus, both ends of the Besseyan scheme fall below the overall figure for frequency of anaerobic species. Analyzing the scheme region by region (Table 1), it is apparent that anaerobiosis is more frequently a character of intermediate evolutionary groups, especially B, than of its extremes. In fact, region B, containing one-third of the orders tested, accounts for 60% of the anaerobes found.

The Angiosperms as a group arose in the Jurassic from an ancestral form not unlike the fossil "seed ferns", and by the mid-Cretaceous had become established as a dominant land vegetation. Thus, there can be no macro-environmental relationship between functional anaerobiosis and the far more ancient reducing atmosphere of the earth. Furthermore, it is reasonable to suppose that the micro-environment of the Jurassic in which seeds germinated is much like that found today.

In terms of flower and fruit morphology, the systematic argument can have no meaning at all, because some of the species whose seeds are most deeply imbedded in fleshy tissues are predominantly aerobic (Rosales, in general) whereas the Liliales, Graminales and Rhoeadales all have little tissue enclosing the seed to act as diffusion barriers.

Thus, there appears to be no obvious environmental or structural factors which could select for the appearance of anaerobiosis. Examining the better-founded results among families (Table 2), one finds anaerobiosis spread over the systematic scheme.

Table 1

Analysis of the Systematic Distribution of Anaerobic Capabilities in Angiosperm Germination

	Region	Number of Orders		Number of Species		Percent Anaerobic
		Anaerobic	Total	Anaerobic	Total	in Region
A	Polypetalous Hypogynous Polycarpellate	3	3	5	30	16
B	Polypetalous Hypogynous Syncarpellate	5	8	34	92	37
C	Polypetalous Epigynous Syncarpellate	2	4	6	32	18
D	Sympetalous Hypogynous Syncarpellate	2	5	8	52	15
E	Sympetalous Epigynous Syncarpellate	2	3	5	35	14
Total		14	23	58	241	24

Table 2

Families in which Ten or More Species were Tested for Anaerobiosis

Family	Species Tested	Germinated	
		Number	%
Compositae	29	4	14
Cruciferae	26	12	46
Cucurbitaceae	10	2	20
Graminae	13	7	54
Labiatae	14	2	14
Leguminosae	15	2	13
Ranunculaceae	10	1	10
Solanaceae	10	1	10
Umbelliferae	10	1	10

Furthermore, if one examines the high-performance Cucurbit family, in which 10 species in 4 genera were tested, only one species, Cucumis sativus showed anaerobic abilities, whereas C. melo and the genera Citrullus, Cucurbita, and Lagenaria did not. Among the cucumber, five varieties gave the following results under identical conditions:

Black Diamond	90%
Long Green	72
Straight 8	79
West Indian Gherkin	62
Wisconsin	29

Even more striking in a quantitative sense was the behavior of the Amaranths. Amaranthus and Celosia both contained anaerobes, but the varieties of Celosia argentea ranged as follows:

Empress	92%
Fire Feather	48
Forest Fire	50
Giant Plume Golden Feather	9
Golden Feather	36
Toreador	33

Finally, as reported earlier, many species will show frequent anaerobiosis, but with low frequency within a given population. The Cruciferae show this kind of behavior with mustards, cabbages, and radishes all germinating but rarely in excess 3-5%.

More data will be accumulated which will permit a return to the phylogenetic problem. The one possible trend toward anaerobiosis,

Ranales ——> Alismatales ——> Liliales ——> Graminales

is inconclusive, lacking greater numbers and samples from adjacent orders. It has recently been noted that already-grown vegetative plants representing Arales, Palmales, Graminales, and Iridales possess good anaerobic performance.

Nevertheless, it is most likely that anaerobic capabilities exist among the Angiosperms as the result of purely random genetic change or have been retained from antiquity in a random fashion. If this is indeed the case, there are good prospects for such capacities in sufficient abundance to provide a large and diversified population of higher anaerobes.

III. GERMINATION IN ATMOSPHERES CONTAINING NITROGEN-OXIDES

In an article in Science (Kiess, C. C., Corliss, C., and Kiess, H. Abstr. Nat. Acad. Sci. Ann. Meeting, Sci. 131, 1319 (1960)), spectroscopic evidence for nitrogen-oxides in the Martian atmosphere is reported, and the assertion made that the presence of toxic nitrogen-oxides constitutes evidence for the non-existence of life. This conclusion has recently been reiterated by Foote (Letters, Sci. 142, 341 (1963)) who used the "non-existence" argument to criticize proposed manned exploration of Mars. In this laboratory, it was observed in early 1962 (Siegel *et al*. Proc. Nat. Acad. Sci. U.S. 48, 725 (1962)) that Winter rye, although capable of slow germination anaerobically, was stimulated by N_2O. It has also been pointed out that NO_3^- serves as a stimulant of anaerobic germination (Quarterly Report One, September 30, 1963).

From the foregoing comments, it is obviously important to establish better understanding of the effects of nitrogen-oxides as atmospheric constituents upon living matter. Even though such compounds are regarded as air pollutants, their alleged toxicity under modified atmospheric conditions requires evaluation by experiment. As the results below demonstrate, ordinary terrestrial seeds are quite capable of germinating in various atmospheres containing nitrogen-oxides, and that other biological materials also show appreciable tolerance to such unusual experimental conditions.

No attempt was made to simulate a Martian environment. Indeed, lacking quantitative data, the nitrogen-oxide content cannot be duplicated. It was decided, however, that the issue in question might be settled by the use of levels of nitrogen-oxides that were (unrealistically) high. Total pressure was maintained at 0.1 $Atm._{Earth}$ or about 75 mm Hg. In one experiment

(Table 3) CO_2 was included, and pH of the aqueous medium was not controlled. Note that individual nitrogen-oxides range as high as 33% by volume, and in a mixed composition, total 50% of the atmospheric gas. This preliminary experiment showed clearly that a capability does indeed exist for seed germination in the presence of nitrogen-oxides. It shows, also, that NO and NO_2 can be toxic under the conditions peculiar to the experiment. In a second run (Table 4), CO_2 was eliminated, the NO and NO_2 pressures were halved, and different species tested. The data show strikingly the stimulating as well as toxic effects of nitrogen-oxides, and also the relatively better performance of some species in pure N_2 as compared to air at 0.1 atm.

The third experiment in this series was more critically designed (Table 5). The low pH of the acidic nitrogen-oxides was eliminated by incubating the seeds on crushed chalk, and the three species tested were good anaerobes.

Only NO_2 alone yielded a poor performance. Germination of rice was enhanced somewhat by N_2O and the N_2-N_2O-NO-NO_2 mixture. Rye was enhanced by N_2O, and sorghum by N_2O, NO, and the mixture.

In various atmospheres, growth as well as germination was observed in experiment 3. The performance of sorghum and rye in NO is especially striking.

Relative to N_2 at 75 mm Hg, the N_2/NO mixture has no effect on rice germination, inhibits rye germination, and stimulates sorghum, whereas post-germination root extension is enhanced by NO in all three species.

In contrast, NO_2 at 12.5 mm Hg in air has no effect on germination percentage, and inhibits root and shoot growth but not severely (Table 6). Thus, there is, not even good reason to accept the thesis that nitrogen-oxides

need be toxic in air, but this provides no basis for drawing conclusions about their effects under other atmospheric conditions.

A few exploratory tests with organisms other than seeds have been conducted. For example, air grown specimens of the plants, Crassula and Euphorbia; larvae, pupae, and adults of Tenebrio molitor; wasps (Vespa communis); and the turtle, Pseudemys scripta-elegans were maintained for about 18 hrs. in 84% N_2O, 3% O_2 and 13% N_2 without harm.

After 24 hrs. in 0.7% O_2, 96.5% N_2O, and 0.7% O_2, Tenebrio larvae, pupae, and adults were still alive, as were a number of succulent plants of the genera Haworthia, Cereus, Faucheria, and Crassula.

Table 3

Experiment 1

Germination in Water

Chamber No.	P (mm Hg) CO_2	N_2	N_2O	NO	NO_2	O_2	Germination (% at 24 hrs.)* pH**	Rye	Rice	Celosia
1	2	60	0	0	0	15	6	76	6	0
2	2	75	0	0	0	0	6	30	3	0
3	2	50	0	25	0	0	2	6	0	0
4	2	50	0	0	25	0	2	6	0	0
5	2	37.5	12.5	12.5	12.5	0	2	0	0	0

* Based upon duplicates of 30-40 seeds each.

** Direct test on water with seeds in chamber.

Table 4

Experiment 2

Germination in Water

Chamber No.	P (mm Hg) N_2	N_2O	NO	NO_2	O_2	Germination (%, 10 Days)* pH**	Bean	Peas	Onion	Tomato	Sorghum
1	75	0	0	0	0	6	0	2	36	2	80
2	60	0	0	0	15	6	0	0	4	10	56
3	62.5	0	12.5	0	0	2	0	0	0	2	0
4	62.5	0	0	12.5	0	2	6	36	2	0	0
5	37.5	12.5	12.5	12.5	0	2	0	0	0	0	0

* Based upon 50 seeds/group.

** Direct test on water with seeds.

Table 5

Experiment 3

Germination on moist crushed chalk

(pH 7)

Chamber	P (mm Hg)				Germination* (%, 5 Days)		
No.	N_2	N_2O	NO	NO_2	Rice	Rye	Sorghum
1	75	0	0	0	64	22	36
2	62.5	12.5	0	0	76	86	50
3	62.5	0	12.5	0	56	8	86
4	62.5	0	0	12.5	2	2	0
5	37.5	12.5	12.5	12.5	86	10	54

* Based upon duplicates of 30-40 seeds each.

Note: At 5 days, some representative growth measurements were:

	In N_2	In N_2O	
	root (mm)	root (mm)	shoot (mm)
rice	2	4	-
rye	1	3	1.5
sorghum	1	1.5	1

	In NO		In N_2O + NO + NO_2
	root (mm)	shoot (mm)	root (mm)
rice	3	-	1.5
rye	9	4	1
sorghum	8	10	

Table 6

Experiment 4

NO_2 in air (5 Days Incubation)

	Air Control	Air with P_{NO_2} = 12.5 mm Hg	Inhibition (%)
Germination %			
Rice	90	86	-
Rye	100	99	-
Sorghum	99	90	-
Root length (mm)			
Rice	5	2	60
Rye	39	20	49
Sorghum	19	15	21
Shoot length (mm)			
Rice	3	1.2	60
Rye	25	9	64
Sorghum	15	5	67

IV. EFFECTS OF CO_2 AND CO_2 + O_2 COMBINATIONS ON SEEDLING GROWTH

Inhibitions of growth and induction of visible injury in general by anoxia are commonly associated with excessive CO_2 production, rather than depletion of oxygen supply. In closed systems and certain micro-environments, accumulation of CO_2 may constitute a distinct biological hazard. The actual extent of CO_2 injury and conditions required for its expression have not been well documented, however. We have observed that plants after many days in high CO_2 (10-50%) retain their normal appearance when 10% or more O_2 is present, and can therefore raise a question about the place of O_2 level in CO_2 damage.

When rye seedlings are grown from seed, their response to each of the factors — CO_2 and O_2 — depends upon the other (Table 7).

Root growth is essentially independent of light or O_2 level at all CO_2 concentrations up to 10%. In 50% CO_2, inhibition is lower in 21% O_2 (balance N_2) with light than in 5% O_2, but similar in darkness at both O_2 levels. Shoot growth in light shows some inhibition at 10% CO_2, but no O_2 dependence. In darkness, CO_2 inhibits only at 50%, and is not affected by O_2 level.

Cucumber seedlings behave quite differently from rye (Table 8). They exhibit a distinct optimum at low CO_2 when in air, but not in 5% O_2.

At low CO_2 levels, which generally do not suppress growth, the respiratory gas exchange of rye seedlings, and their growth were compared as CO_2 was varied (Table 9). The results of this study confirmed the lack of an inhibiting effect of CO_2 at 5% CO_2, but showed quite strikingly that respiration could be suppressed severely without inhibiting root or shoot elongation.

Table 7

Effect of CO_2 on Rye Seedlings

at Two O_2 Levels

CO_2	In Light*		In Darkness	
%	21% O_2**	5% O_2**	21% O_2	5% O_2
	a. Root Growth (cm)			
0	6.8	6.4	6.5	5.7
1.7	7.6	5.7	8.8	6.9
10	5.7	5.7	6.4	5.7
50	1.7	0.3	0.4	0.2
	b. Shoot Growth (cm)†			
0	7.0	6.1	4.5	5.1
1.7	7.5	7.4	5.6	5.2
10	4.7	4.8	5.2	4.9
50	1.5	0.3	0.3	0.2

* 250 f.c. soft white.

** Balance N_2.

† Based upon measurements of 25-30 seedlings.

Table 8

Effect of CO_2 on Cucumber Seedlings

in Darkness at Two O_2 Levels

CO_2 %	Root† (cm) 21% O_2*	Root† (cm) 5% O_2*	Shoot† (cm) 21% O_2	Shoot† (cm) 5% O_2
0	3.6	3.7	0.4	0.8
1.5	5.1	3.1	1.5	0.5
10	3.4	3.1	0.6	0.5
50	0.5	0.2	-	-

* Balance N_2.

† Based upon 25-33 Seedlings/group.

Table 9

Respiratory Gas Exchange and Growth in Rye

Seedlings in Air at Various CO_2 Levels*

CO_2 %	Respiration Rate (cm^3/ seedling/24 hr.) O_2	Respiration Rate (cm^3/ seedling/24 hr.) CO_2	Growth cm in 3 days Root	Growth cm in 3 days Shoot
0	-0.72	0	3.6	2.1
0.005	-0.60	+0.66	4.6	2.4
0.03	-0.48	+0.63	5.0	2.7
0.05	-0.48	+0.63	4.7	2.7
0.10	-0.48	+0.48	3.8	2.5
1.0	0	+0.33	5.3	2.7
5.0	-	+0.09	4.5	2.9

* Based upon 25 seedlings/group.

V. GERMINATION AND GROWTH IN ATMOSPHERES CONTAINING VOLATILE ORGANIC COMPOUNDS

Although most atmospheric studies in this laboratory have been concerned with simple inorganic compositions, there are circumstances under which the effects of organic substances could be of some importance. Thus, it is possible that certain simple organic compounds could be present in extra-terrestrial planetary atmospheres. Sinton has produced tentative evidence for an organic atmospheric component over the dark areas of Mars, possibly acetaldehyde; and methane and formaldehyde are also conceivable constituents. Beyond their natural occurrence, volatile metabolites of relatively high density could be sustained in a ground level micro-environment provided that relatively low temperatures and minimum air movements prevail. Finally, in closed biological systems, organic compounds could be introduced inadvertently as atmospheric contaminants.

In the following experiments, the atmospheric composition has been calculated as if the organic constituent were wholly in the gas phase. Experiments were carried out in 4 liter vessels with about 4 cm. of moist perlite substratum. The germination of rye in experimental atmospheres (Tables 10 and 13) and the grow ı of air-grown (5 day old) rye and cucumber during 3 days in test conditions (Tables 11 and 12) are included.

In rye, the elongation of the primary leaf was measured initially (L_o) and after 3 days (L_f). Rye is comparatively uniform in its growth, as individual measurements (Table 11) show. Defining the "normal" range as $\overline{\Delta L} \pm 3\sigma$, control and test populations may be compared.

Although methanol inhibits growth, 8 out of 10 plants actually fall within the control growth range. Formaldehyde, although even more

Table 10

Germination of Winter Rye in Atmospheres Containing Volatile Organics (10% O_2 - 40% N_2 - *ca* 50% X) at P = 760 mm

Compound X	Germination (%) after 72 hrs.*
None	96
CH_3OH	92
CH_2O	40
n-C_3H_7OH	88
n-C_5H_{12}	80
iso-C_5H_{12}	12
n-C_7H_{16}	0

* Based upon 30-40 seeds.

Table 11

Growth of Winter Rye in Atmospheres Containing

Volatile Organics (10% O_2- 40% N_2- ca 50% X)

Compound X		Plant No. 1	2	3	4	5	6	7	8	9	10
	L_o	2.1	2.3	2.7	2.0	3.2	2.8	2.4	2.5	3.2	3.0
None	L_f	9.2	10.9	12.1	9.5	8.5	10.4	8.5	12.0	9.6	12.7
	ΔL	7.1	8.6	9.4	7.5	5.3	7.6	6.1	9.5	6.4	9.7
		$\overline{\Delta L}$ 7.7 cm $\pm$ 1.5 cm									
		$\overline{\Delta L} - 3\sigma = 3.2$					$\overline{\Delta L} + 3\sigma = 12.2$				
	L_o	2.5	3.0	3.5	2.8	3.8	3.2	2.5	2.2	5.2	3.8
CH_3OH	L_f	5.4	7.5	8.7	6.5	6.3	7.4	5.8	7.0	9.5	8.4
	ΔL	2.9	4.5	5.2	3.7	2.5	4.2	3.3	4.8	4.1	4.6
		$\overline{\Delta L}$ 4.0 cm $\pm$ 0.9									
	L_o	2.1	2.1	2.5	2.6	2.9	2.6	2.2	3.0	2.8	2.8
CH_2O	L_f	6.8	6.2	7.3	2.8	4.5	6.5	2.8	3.5	4.1	3.8
	ΔL	4.7	4.1	4.8	0.2	1.6	3.9	0.6	0.5	1.3	6.0
		$\overline{\Delta L}$ 2.3 cm $\pm$ 2.1									
	L_o	3.0	3.5	3.6	3.2	3.2	2.5	4.0	2.6	3.2	2.4
n-C_3H_7OH	L_f	3.8	3.7	3.8	4.4	4.1	3.7	4.5	4.0	5.5	5.0
	ΔL	0.8	0.2	0.2	0.8	0.9	1.2	0.5	1.4	2.3	2.6
		$\overline{\Delta L}$ 1.1 cm $\pm$ 0.8									
	L_o	2.9	2.8	3.0	2.7	2.5	3.5	2.5	2.7	2.4	2.5
n-C_5H_{12}	L_f	3.7	2.8	3.2	3.2	3.3	3.5	2.5	3.2	3.0	3.0
	ΔL	0.6	0	0.2	0.5	0.8	0	0	0.5	0.6	0.5
		$\overline{\Delta L}$ 0.4 cm $\pm$ 0.2									

Table 11 (Contd.)

Compound X		Plant No. 1	2	3	4	5	6	7	8	9	10
	L_o	2.7	2.3	3.0	3.1	3.0	2.4	2.1	3.3	2.8	3.1
iso-C_5H_{12}	L_f	3.2	2.5	3.4	3.5	3.0	3.0	2.7	3.6	3.1	3.5
	ΔL	0.5	0.2	0.4	0.4	0	0.6	0.6	0.3	0.3	0.4
		$\overline{\Delta L}$ 0.4 cm $\pm$ 0.2									
	L_o	2.6	2.7	3.2	2.6	3.8	2.8	3.1	2.7	2.8	2.1
n-C_7H_{16}	L_f	3.1	3.5	3.2	3.0	3.8	3.0	3.5	3.1	3.0	3.1
	ΔL	0.5	0.6	0	0.4	0	0.2	0.4	0.4	0.2	0.4
		$\overline{\Delta L}$ 0.3 cm $\pm$ 0.6									

Table 12

Growth of Black Diamond Cucumber in Atmospheres Containing Volatile Organics (10% O_2 - 40% N_2 - ca 50% X)

Compound X	Growth - 72 hrs. Hypocotyl ΔL_{cm}	Bud ΔL_{cm}
None	0.42	0.21
CH_3OH	0.65	0.15
CH_2O	0.32	0.22
n-C_3H_7OH	0.29	0.06
n-C_5H_{12}	0.23	0.07
iso-C_5H_{12}	0.12	0.13
n-C_7H_{16}	0.15	0.05

Table 13

Comparative Effects of Organic Vapors

on Rye and Cucumber

Compound	Growth Response (Δ% relative to Control)			
	Rye Germination	Rye Leaf Growth	Cucumber Hypocotyl Growth	Cucumber Bud Growth
CH_3OH	- 4	-48	+55	-29
CH_2O	- 58	-70	-24	+ 5
n-C_3H_7OH	- 8	-86	-30	-71
n-C_5H_{12}	- 17	-94	-45	-67
iso-C_5H_{12}	- 88	-94	-71	-38
n-C_7H_{16}	-100	-96	-64	-76

inhibitory, still allows half of the test plants to grow relatively well. The remaining compounds, n-propanol, the pentanes and n-heptane all suppress growth severely in all individuals.

The differential sensitivity of the test pl nts to the compounds used is reflected by changes in the "coefficient of variability" (Pearson's coefficient $C = 100\ \sigma/\ \bar{X}$):

Control	C = 20%
CH_3OH	21
CH_2O	100
n-C_3H_7OH	72
n-C_5H_{12}	75
iso-C_5H_{12}	43
n-C_7H_{16}	48

The responses of cucumber were indicated both by stem (hypocotyl) and leaf (bud) growth (Table 12).

Altogether, four growth systems can be examined comparatively (Table 13). The limiting processes are:

rye germination	release of dormancy ("activation")
rye leaf extension	cell division
cucumber hypocotyl extension	cell enlargement
cucumber bud growth	cell division

No uniform patterns of response can be established, even for the same elementary process - cell division - in the rye leaf and cucumber bud. Three of the four test systems can distinguish the pentane isomers, but their relative toxicity differs widely.

It appears that the responses to this small series of organic vapors is rather individualistic, in fact far more so than would have been anticipated. The physiological and biochemical value of this kind of comparative study is, accordingly, greatly enhanced.

One final observation has been made with methane atmospheres (Table 14). Germination in atmospheres rich in methane, but also containing some O_2 is essentially identical with germination and growth in 5% O_2 + Argon. More interesting, in 100% CH_4, rye can still initiate germination, even if slowly. Clearly hydrocarbon gases are not inherently toxic, even if some of them have specific effects.

Table 14

Growth of Rye in Methane Compositions

Vessel	Composition (Vol %)			Germination	Root	Shoot
No.	O_2	Ar	CH_4	%	cm	cm
1	5	95	0	94	2.3	0.9
2	5	70	25	97	2.2	0.8
3	5	45	50	94	2.3	1.0
4	0	0	100	52	0.2	0

VI. THE LOW TEMPERATURE-HYDROGEN INTERACTION

Most seeds fail to germinate if, after imbibition, they are subjected to temperatures low enough to freeze the embryonic tissues. After emergence of the radicle, sensitivity to cold is commonly increased. There are, however, striking exceptions among common species which can tolerate temperatures well below the tissue freezing point. On a 24-hr. cycle in which -30°C was held for 8 hrs., onion, carnations, radish, some Graminae, and other species were able to germinate (Table 15). Radicles which were "hard-frozen" and brittle during the cold phase resumed a normal condition during the 16-hr. warm phase of the daily cycle. On this temperature regime, inter-varietal differences were quite evident among carnations, radishes, and cucumbers. Inter-specific differences were evident between *Dianthus barbatus*, a small ornamental, and *D. caryophyllus* (the carnation), and inter-generic differences among the grasses, Briza, rice, and rye. Of the 13 species and varieties which germinated on the short sub-freezing cycle, 5 survived a cycle consisting of 16 hrs. at -30°C and 8 hrs. at +22°C, but only Winter rye performed relatively well.

The unusual tolerance to low temperatures exhibited by some species suggests that such stresses evoke appreciable physiological changes. The study of low temperature physiology and biochemistry is only in its preliminary phase, but has already revealed important differences. At 22°C, a variety of seeds can germinate in H_2, but more slowly or less completely than in air (Table 16). Extreme examples are highly aerobic lettuce, which fails completely to germinate in H_2, and broccoli which shows only marginal germination.

Table 15

Some Comparative Tolerances Toward Diurnal

Temperature Extremes in Germination

Species	Germination (%) after 5 Days at -30°C 8 hrs. + 22°C 16 hrs.	-30°C 16 hrs. +22°C 8 hrs.
Onion (Yellow Globe)	100	12
Dianthus barbatus	80	0
Carnations (D. caryophyllus)		
var. Chabands Giant	5	5
var. Bright Scarlet	20	3
Radish		
var. Crimson Giant	30	0
var. Round Black Spanish	73	0
var. Scarlet Turnip White Top	100	10
Cucumber		
var. Black Diamond	84	0
var. Straight 8	0	0
var. Long Green	0	0
Briza maxima	90	0
Oryza sativa var. fatua	66	0
Winter rye	90	20
Oxalis sp.	80	0
Parsley	39	0

Table 16

H_2-Stimulated Germination at Low Temperatures

	6°C		22°C	
	Air	H_2	Air	H_2
Barley	97	68	97	21
Winter rye	90	98	95	56
Winter wheat	15	30	89	25
Cucumber	0	28	97	35
Kale	10	27	--	--
Broccoli	3	12	90	5
Radish ("Icicle")	0	33	95	20
Radish ("Champion")	0	48	100	15
Lettuce ("Grand Rapids")	2	40	97	0

When, however, the same species were incubated at 6°C in air all but barley and Winter rye were severely or completely inhibited. In every instance of severe or complete inhibition in air, the substitution of H_2 led to enhancement of germination. Thus the nature of the response to hydrogen appears to reverse itself at 6°C relative to 22°C, clear indication of an altered physiological state.

An additional indication of the alteration in seed physiology at low and high temperatures was obtained with Winter rye and selected metabolic poisons. For example, sodium azide and iodoacetamide at 5×10^{-4}M have no effect on germination at constant 22°C, but inhibited germination completely when 16 hrs. or more per 24 hrs. were at 4°C. Sodium arsenate inhibited under both conditions, but was also somewhat more active at low temperature.

VII. FURTHER SELECTION STUDIES

Argon was originally suggested as a selective medium for study of anaerobiosis. Results summarized in Quarterly Report One indicate that the selection pressures imposed by argon may be excessively rigorous for practical purposes. Cell division was limited in argon and germination consisted of morphoses of questionable significance to the control problem, i.e., identification of genotypes capable of growth and development under anoxia. Hydrogen, though capable of supporting cell division in the more highly anaerobic types, was apparently incapable of elucidating degrees of anaerophilia as measured by germination. Furthermore, argon retarded growth rates among certain types of general interest — specifically leguminous species. Hence emphasis has shifted to the possible use of helium and micro-aerobic environments as selective media.

Preliminary results with helium indicate that this gas may have considerable utility to the present program. Seeds of each of two varieties from three of the more anaerophilic species were placed in chambers filled with air or helium. Samples were removed after 3 days and 7 days and their germination recorded. Survival was recorded 3-4 days later. Percent germination was significantly greater in rice than in rye or wheat. Varietal differences were also evident in rice, Calora > Patna, and in rye, 3915 > 816. The apparent equivalence of rye and wheats may be illusory in that development apparently proceeds farther and is better regulated in the former species. Anaerobic "germination" of wheats consists of little more than extrusion of an amorphous mass of tissue. Differences in survival between varieties and treatments, according to durations of exposure, indicate that He, unlike H_2 and Ar, may be toxic. Survival of rye #816 and Genessee wheat

was reduced 38 and 19% respectively by 7 days in helium.

One percent oxygen in helium was used to investigate potentialities of micro-aerobic environments as selective media for Winter rye. Although 1% oxygen is less than is required to sustain normal oxidative metabolism, germination was initially more rapid than in air. After 18 hrs. incubation, germination in 1% O_2 (76%) approached that of the control and exceeded that in helium (28%) ca. three-fold. Differences, although diminished, still persisted at 69 hrs. (Table 18) when the treatment phase of the study was terminated. Qualitative differences between treatments were even more striking. Seedling height, root growth and branching were only slightly reduced in 1% O_2 whereas seedlings grown in helium were comparable to 20 hr. controls.

Further tests were conducted with rice (Calora), rye (3915) and wheat (Genessee) to (a) better evaluate 1% oxygen as a selective sieve for anaerophilia; (b) to further examine the apparent helium toxicity. Germination and survival were comparable for all three types in air and 10% O_2 + 99% He (Table 19) but evaluation of selective properties of 1% O_2 may still be possible through quantitative comparisons of preserved specimens. The marked reduction in survival of Genessee wheat in helium confirms previous indications of helium toxicity in this species. The fact that survival was reduced, albeit less markedly, in 1.0% oxygen + 99% helium suggests that lethality may be an effect of helium *per se* rather than enhanced sensitivity to atmospheric oxygen.

Table 17

Responses of Cereals in Air and Helium

		Percent Germination			
		3 Days		7 Days	
		Air	Helium	Air	Helium
Wheat	Pennoll	96*	65	94	64
	Genessee	92	66	92	75
Rye	3915	100	73	99	72
	816	97	46	95	50
Rice	Calora	100	98	97	99
	Patna	87	87	88	83

		Percent Survival			
		3 Days		7 Days	
		Air	Helium	Air	Helium
Wheat	Pennoll	84	90	97	88
	Genessee	92	83	96	78*
Rye	3915	94	98	94	94
	816	96	85	97	60*
Rice	Calora	98	98	96	98
	Patna	77	86	89	80

* Percentages based upon at least 100 seeds.

Table 18

Effect of 69 hrs. in Micro-aerobic and Anaerobic Environments on Germination of Winter Rye (#3915)

	Air	1% O_2 + He	Helium
Percent Germination	98	97	68
Percent Survival	95	91	90

Table 19

Toxic Effects of Helium

Percent Germination		Air	1% O_2 99% He	He
Rye	3915	100*	97	44
Rice	Calora	100	97	96
Wheat	Genessee	98	86	73

Percent Survival - 3 Days	Air	1% O_2 99% He	He
Rye	99	95	99
Rice	97	99	100
Wheat	94	91	93

Percent Survival - 7 Days	Air	1% O_2 99% He	He
Rye	98	96	89
Rice	98	97	96
Wheat	97	84	66*

* Percentages based upon at least 100 seeds.

VIII. OTHER AREAS OF INVESTIGATION AND ACTIVITY

A. Further experiments with the ultracentrifuge are now in progress. Although these results are incomplete, they show that seed pre-treatment, even at accelerations of 140,000 g while capable of inhibiting their subsequent germination, but are far from severe in their effects.

B. A continuation of the study of phosphate-oxygen relations has now encompassed 6 different varieties of rye. With respect to the effect of the phosphate on germination, for example, the following comparisons may be made:

In air, no effects of significance have been noted.

In 97% N_2 + 3% CO_2, optimum orthophosphate concentrations (in moles/L) for germination were:

	at 0.1 atm.		at 1.0 atm.
Rosen	0.005		0.01
Funk Bros.	0.01		0.025
Rudy Patrick	0.01		0.01
Foster Kendall	0.005		0.01
Ouren	0.01	equal from	0.0025-0.025
Barteides	0.0025		0.01

In general, at 1 atm. the phosphate concentration is shifted to a moderately higher value.

Salinity studies are also continuing with rye varieties, and will be extended to other promising species such as rice and sorghum.

C. The effect on germination and seedling growth of all-visible light sources differing in actual spectral energy distribution is being tested. Results thus far show pronounced effects of green-rich illumination on some species.

D. We also wish to call attention to the talk entitled "Experimental Approaches to Martian Biology" given by S. M. Siegel at the Food Science Conference held November 19-21, 1963 at the Army Natick Laboratories, Natick, Massachusetts. This meeting was sponsored jointly by the National Research Council, the National Academy of Sciences, the U. S. Army Quartermaster, and the Quartermaster Research and Development Associates. The script for this talk received prior approval by Mr. R. T. Mittauer, Public Affairs Officer, National Aeronautics and Space Administration, Washington, D. C.